Technical Report No. 50

GENETIC STRUCTURE AND VARIABILITY IN TWO SPECIES OF ENDEMIC HAWAIIAN *DROSOPHILA*

William W. M. Steiner

Department of Genetics and Development
515 Morrill Hall
University of Illinois
Urbana, Illinois 61801

Hampton L. Carson

Department of Genetics
University of Hawaii
Honolulu, Hawaii 96822

ISLAND ECOSYSTEMS IRP

U. S. International Biological Program

December 1974

ABSTRACT

Two endemic sympatrically occurring Hawaiian Drosophila species found in closely adjacent Kipuka Ki and Kipuka Puaulu on the island of Hawaii have been examined at about 20 electrophoretic loci for the extent of their genetic variability. In D. mimica, 47.6% of such loci were polymorphic, with individuals polymorphic at ≈ 19% of their loci on the average. For D. engyochracea, these figures are 30% and ≈ 12%. Levels of variability appear to be higher in Kipuka Ki populations of both species. In addition, higher numbers of alleles are observed in D. engyochracea than are expected from predictions based on the neutral gene hypothesis. The evolutionary advantages of such high levels of variability are discussed and the suggestion is made that the genetic variability in these species serves some adaptive function.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

INTRODUCTION

The Hawaiian Drosophila are a unique group of organisms particularly known for their rapid evolution and speciation. These flies have been a major source of study since the early 1960's with most of the emphasis placed on bringing to light phylogenetic relationships. These relationships have been based on a variety of fields of investigation including information gained from studying their cytogenetics, internal and external morphology, ethology, ecology, ovarian transplantation and biochemical genetic polymorphisms. This work has recently been reviewed by Carson et al. (1970) who also point out the major factors possibly contributing to their evolution. They cite a paucity of feeding and breeding sites which resulted in the evolution of a low reproductive rate and consequent small population sizes. They believe that there were infrequent but repeated migrations between islands resulting in effective isolating barriers. Volcanic and meteorological action resulted in fractionation of gene pools. There was a pronounced evolution of lek behavior and specialized food sources such as the leaves of different plant species and even the eggs of spiders were adopted.

All of the above must have some impact on the genetic structure of populations of these drosophilids, but these particular relationships remain largely uninvestigated. Small population sizes, for example, coupled with repeated fractionation of gene pools, can lead to reduced genetic variability in natural populations, particularly where the population originated via a single colonizer or founding event. On the other hand, consistent migration between populations should be a method of maintaining genetic variability and common genetic bonds between populations. Prolonged maturation time, which also occurs in the Hawaiian Drosophila, should serve a similar function in that the chance for mating between sibs is reduced during possible dispersive stages of adulthood. This should effectively reduce any effect due to inbreeding, although it is possible that inbreeding depression may already be adjusted for in such populations (e.g., see Stone et al., 1966). Although speculative, it is feasible that lek behavior may also have evolved as a mechanism to insure generation of heterozygosity.

The question of the extent of genetic variability in small and isolated populations is important in the Hawaiian Drosophila because of the above considerations. The classical approach has considered genetic variability as being important in speciational events, first because the accumulation of genetic differences in isolated populations may be a first step toward speciation and second because genetic variability is thought to underlie adaptive genetic shifts envisioned to occur in

populations responding to environmental fluctuations. Little investigation has been done, however, into the extent of genetic variation in insular gene pools. At the level of the chromosome, Heed and Russell (1971) compared inversion variability in eight species of the Drosophila cardini group from the Greater and Lesser Antilles with eight South American species. Of the 162 inversions present across all species, only 15 occurred in the insular species. Ayala et al. (1971) found a similar situation for chromosome variability in Drosophila willistoni. Here, nine South American (continental) populations contained approximately 4.1 times as many inversions as did nine West Indies populations. At the genic level, however, genetic heterozygosity levels were similar although island populations had slightly lower levels of individual variability (18.4% of loci heterozygous in continental populations versus 16.2% in island populations). Lower genic variability has also been observed in an isolated but marginal population of Drosophila pseudoobscura near Bogota, Columbia (Prakash et al., 1969).

In two studies conducted on two species of Pacific island drosophilids, Stone et al. (1968) and Johnson et al. (1969) found polymorphism present at the enzyme loci they examined. Levels of genic variability were fairly high in both with up to 36.5% of the genome of a nasuta-complex species and 24.8% of that of D. ananassae being variable (Table 1). The estimates, based only on 3 loci for the first species and 4 for the second, appear to be in line with genetic variability observed in other continental species. Although possibly biased because of the low number and the type of loci examined, a more in-depth study by Johnson (1971) supports this finding and suggests that the reason for such high levels in these semi-isolated populations may be due to both huge population sizes and extensive inter-island migration. The above species appear to have a more cosmopolitan nature, and though isolated to some extent, have been experiencing recurrent migration over wide geographical areas.

Studies on genic variability in endemic and isolated island populations are especially limited. In the Hawaiian Drosophila, Carson et al. (1967) has shown that, despite pronounced morphological diversity across hundreds of species, strong karyotypic stability prevails. In a close examination of 22 species, no changes in metaphase chromosome number were detected and the giant polytene chromosomes had undergone only moderate reorganization by inversions. In many instances formation of two or more species had occurred with no detectable change in the giant chromosomes. Carson et al. conclude that speciation in these animals had to be based entirely on submicroscopic, mutational (genic) changes.

Population differences within some of these species are evident at the

TABLE 1. Estimates of genic heterozygosity in island populations of 2 species of Drosophila (calculated from the data of Stone et al., 1968 and Johnson et al., 1969).

	Samoan Populations	Fijian Populations
D. spinofemora	36.5%	26.9%
D. ananassae	11.7%	24.8%

chromosomal level, however. Carson and Sato (1969) examined 4th chromosome inversion frequencies in several populations each of D. bostrycha, D. disjuncta and D. grimshawi. They found that intraspecific populations separated across even short distances demonstrated evidence of being genetically distinct, despite the fact that one inversion polymorphism is common to both D. bostrycha and D. disjuncta. Random genetic changes associated with gene pool fractionation and followed by selection on the resulting differing gene pools was thought the most likely candidate leading to such changes, although ecotypic responses to slightly differing niches could not be ruled out as a primary factor.

It is apparent that what is needed here are answers to questions concerning the extent and the adaptive nature of genic diversity in the Hawaiian Drosophila. In an attempt to answer the first question, Rockwood et al. (1971) examined 78 species for their phylogenetic relationships as well as the extent of genic diversity at the biochemical level. Although a wide range of variability was found in individual heterozygosity (h = 0-88%) and average heterozygosity (0-39.8%, with 2 species monomorphic), the study suffered the drawback of having mostly small sample sizes with less than eight loci examined in many cases. A more in-depth study by Rockwood (1969) exposed a time-dependent trend at a three-allele acid phosphatase (ACPH) locus in D. mimica. Using starch gel electrophoresis, the homozygous fast electrophoretic variant showed a significant decrease in frequency from 28% in December to 4% in May in the Kipuka Ki population (Table 2). A similar trend observed in Kipuka Puaulu was not significant. The trend was interpreted as an adaptive response to some unknown factor(s) in the environment. In a more defined study, Johnson et al. (1975) surveyed the D. planitibia subgroup for enzyme variability. The range of variability was 3.2% - 12.8% for some 12 gene loci. It appeared, however, that genetic variability was affected by the kind of founding and speciation event a particular species had undergone. Further evidence that gene pool fractionation can effect the type of genic variability present was given by Steiner et al. (1973).

From the preceding review it is apparent that little information is available on the genetic structure of isolated and endemic insular species. This is particularly important in the endemic Hawaiian fauna where instances of extensive speciation have occurred. The relationships of the genetic variation present to environmental variability is of special interest because in areas of high environmental predictability such as those occupied by the Hawaiian Islands, a lesser degree of genetic variability may be expected with higher species diversity (i.e., see Slobodkin and Sanders, 1969; Beardmore and Levine, 1963). The effects of gene pool fractionation,

Table 2

Summary of gene frequency data from previous studies for the Acid Phosphatase (ACPH) locus in D. mimica and the Esterase-B (EST-2) and Esterase-C (EST-3) loci in D. engyochracea (after Rockwood, 1969 and Rockwood et al., 1971).

Locus	Site	Collection Date	N	Allele 1	2	3	Homozygote 1-1 Frequency
ACPH	KK‡	12.1.67	53	.491	.491	.019	.280
		2.1.68	84	.316	.631	.054	.155
		3.1.68	94	.277	.660	.064	.060
		6.1.68	87	.356	.609	.034	.040
	KP‡	12.2.67	78	.365	.583	.051	.115
		2.2.68	81	.346	.605	.049	.185
		3.2.68	67	.299	.672	.030	.080
		6.2.68	59	.322	.585	.093	.136
EST-2	KK		21	.476	.429	.053	--
	KP		113	.518*	.429	.053	--
EST-3	KK		24	.813	.188	NP	--
	KP		92	.717	.239	.043	--

See Table 4 for abbreviations

* Homozygote 1-1 in excess, class not in equilibrium.

NP not present

‡ KK = Kipuka Ki; KP = Kipuka Puaulu

the founder effect, and adaptive responses on levels of genetic variability already affected by a predictable environment are experimentally undetermined.

In a series of studies we have attempted to throw some light on the above problems. Changes in genetic variability, environment and a quantitative physiological trait were investigated over time in two sympatric and endemic Hawaiian Drosophila species. This paper describes the extent and nature of the genetic variability present in these isolated populations.

DESCRIPTIONS OF STUDY SPECIES

Drosophila mimica and D. engyochracea were chosen as study species because they were easily available for collection, maintained large population sizes the year round, were susceptible to electrophoretic analysis, occurred in a site where accurate and concurrent meteorological data was being collected and had much information available on their ecological and genetic backgrounds. In addition, chromosomal inversion polymorphism is restricted in D. mimica and appears to be absent in D. engyochracea. Both species are fairly large and long-lived and D. mimica is easily culturable under laboratory conditions.

Both species are found at Kipuka Ki (KK) and Kipuka Puaulu (KP) on the island of Hawaii (Figure 1). These are two forested pockets of vegetation which were surrounded and isolated by a lava flow approximately 2,100 years ago. The Kipukas are about 1.5 km apart and located about 4 km northwest and upslope of Kilauea Crater. A description of these sites, including plant profiles, is given in Mueller-Dombois and Lamoureux (1967). Generally, Kipuka Puaulu appears to show greater biotic diversity.

D. mimica and D. engyochracea appear essentially confined to these Kipukas. Scattered reports exist for their occurrence at other sites on the island of Hawaii (H. L. Carson, Collection Records, Hawaiian Drosophila Project, University of Hawaii, Honolulu). The species status of these specimens have not been confirmed and at any rate, they are extremely rare elsewhere. There seems to be no evidence for gene flow between the above Kipukas for D. mimica (S. Johnston, personal communication), and it appears that the populations are very strongly isolated.

Descriptions of both species can be found in Hardy (1965), while phylogenetic relationships for D. engyochracea can be found in Kaneshiro (1969) and Carson et al. (1970), and for D. mimica in Yoon et al. (1972). Information on their mating behavior is available in Spieth (1968) and their ecology from Heed (1968), Montgomery

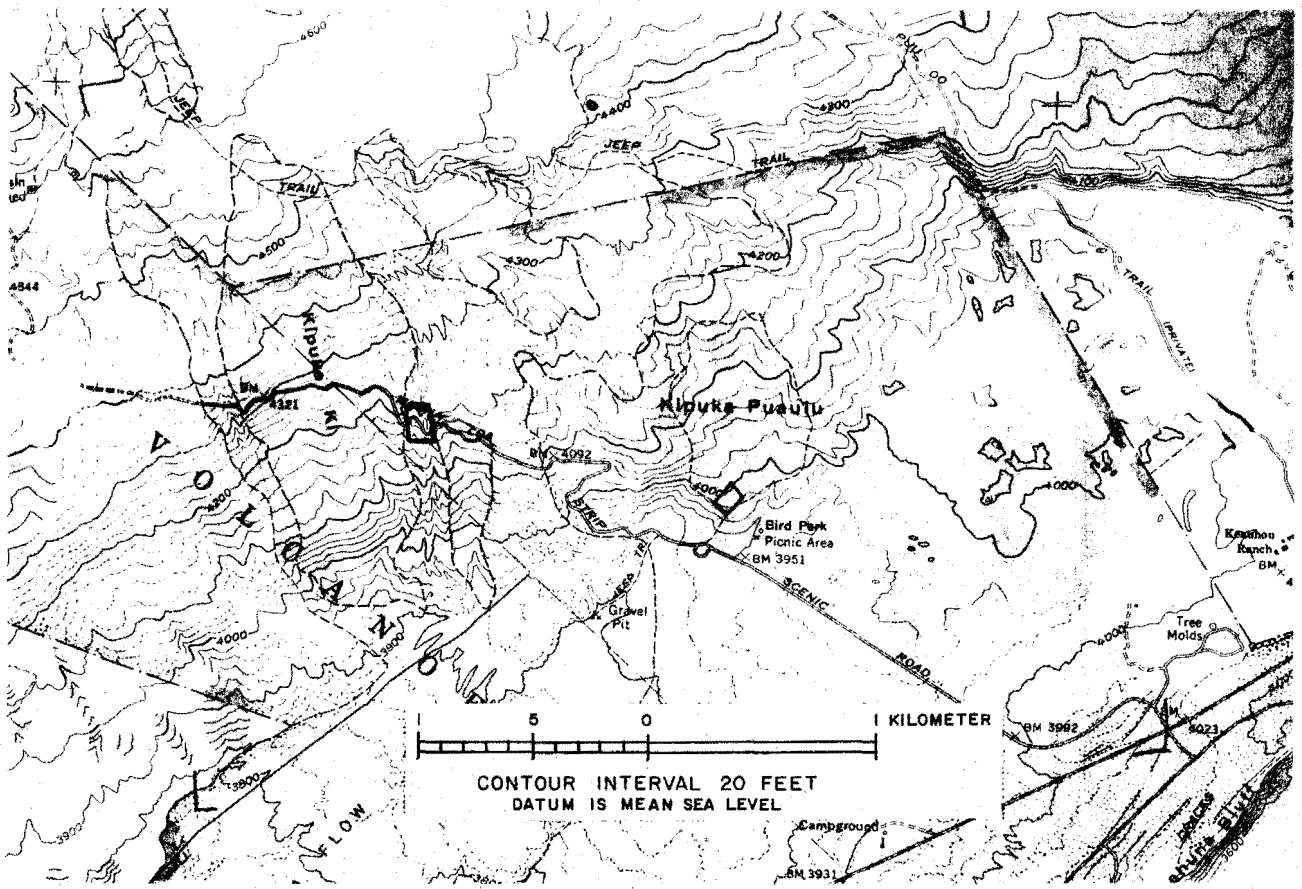


Figure 1. Location of collecting sites (small rectangles) in Kipuka Ki and Kipuka Puaulu, Volcano National Park, Hawaii.

(1972), Kambysellis and Heed (1971), Kambysellis (1974) and Richardson (1974). Genetic studies for both species are found in Rockwood (1969) and Rockwood et al. (1971); the allozyme data from these papers are summarized in Tables 2 and 3. No evidence for population differences is indicated for D. mimica, although it is evident, as previously discussed, that temporal shifts exist involving the frequency of the homozygote for the electrophoretically fast migrating allele at the acid phosphatase (ACPH) locus. Differences in gene frequencies and number of alleles present at two esterase (EST-B, EST-C) loci in D. engyochracea suggest possible population differentiation (Table 2). No studies of gene flow between the Kipukas exists for this latter species and the number of genomes sampled is small.

The data in Table 3 suggest that reduced levels of variability may be present in Kipuka Puaulu populations of D. engyochracea but probably not for D. mimica. This would be consistent with what is known of the ecology of these species since Kipuka Ki may be a more xeric environment. D. engyochracea can normally be found sitting on the exposed branches or the boles of Sapindus, Pipturus and Coprosma trees during warm parts of the day while D. mimica seeks shelter and buffers itself from the environment in the cooler, moister leaf litter on the ground.

MATERIALS AND METHODS

An experimental design was used which was intended to maximize information output and correlation (Figure 2). Collections were made, after preliminary trials, every three months in a staggered fashion for each species. For D. mimica, collection was made by net sweeping over leaf and Sapindus saponaria berries with a 12" insect net. For D. engyochracea, collection was made by setting out baits of yeasted Gerber's baby food of banana-pineapple variety. Fleischmann's yeast was mixed with the bait one day prior to use. Baits were placed approximately chest high on the boles of Sapindus saponaria trees and on horizontal branches of surrounding trees, particularly Coprosma cymosa and Pipturus hawaiiensis, two native Hawaiian trees. Collections were generally continuous from between 8:30 a.m. to 6:30 p.m. with a slack period of low activity between 11:00 a.m. and 2:00 p.m. a usual event.

Flies were placed on sugar media (Spieth, 1966) over ice packs in insulated metal containers for transport back to the laboratory of Dr. H. L. Carson, Department of Genetics, University of Hawaii, Honolulu, where further analysis was undertaken.

Table 3

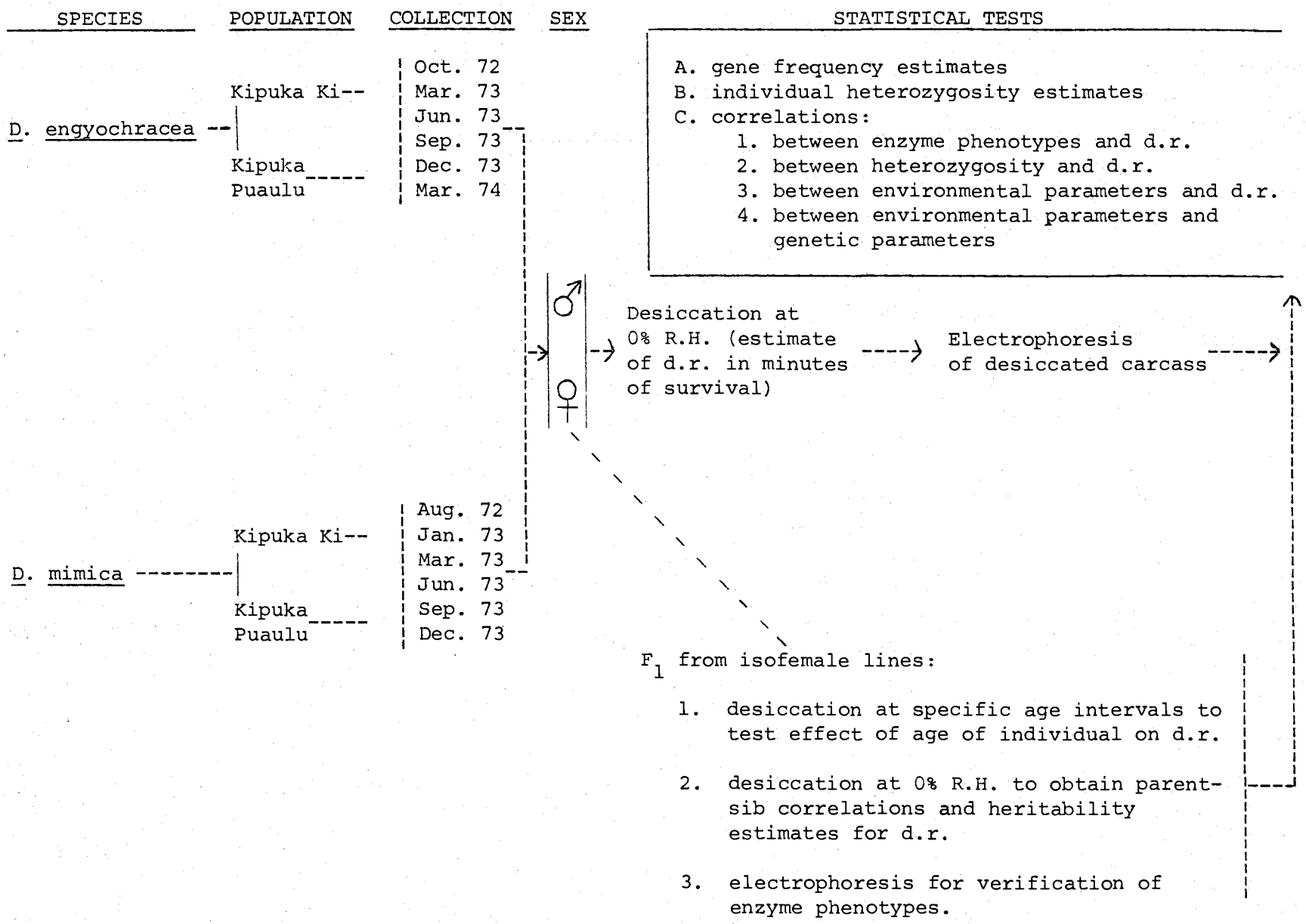
Summary of heterozygosity estimates from previous studies for two Hawaiian *Drosophila* species (after Rockwood, 1969 and Rockwood et al., 1971).

	D. mimica				*D. engyochracea*			
	No. of Alleles		h		No. of Alleles		h	
Locus	KK	KP	KK	KP	KK	KP	KK	KP
ADH	1	1	--	--	1	1	--	--
α-GPDH	1	1	--	--	1	1	--	--
MDH	1	1	--	--	1	1	--	--
ACPH	3	3	.505	.500	ND	ND	--	--
APH	6	6	.766	.723	ND	ND	--	--
EST-2	4	4	.177	.276	3	3	.480	.380
EST-3	ND	ND	--	--	2	3	.290	.470
ODH	2	2	.360	.382	1	1	--	--
LAP-2	2	2	NA	NA	2	2	NA	.500
Population $\bar{H}$ =			.258	.269			.212	.170

See Table 4 for abbreviations

ND = No data
NA = Not analyzed
M = Monomorphic
$h = \frac{\Sigma \text{ No. of heterozygotes at a locus}}{\text{No. of individuals assayed}}$
$\bar{H} = \frac{\Sigma \text{ heterozygotes (all loci)}}{\text{Total no. of individuals assayed x total no. of loci}}$

Figure 2. The general experimental design in an investigation of possible relationships between a quantitative trait (desiccation resistance, d.r.), enzyme polymorphisms and specific environmental parameters (temperature, relative humidity (R.H.) and rainfall).



Electrophoresis was done as previously described (Steiner and Johnson, 1973) after obtaining an F_1 generation from the females and after subjecting males and females to a desiccation stress test. This enabled the determination of both gene frequency data and individual phenotype profiles for subsequent stress-phenotype correlation analysis (to be discussed in a later paper). Examination of unstressed controls on each gel indicated that there were no observed effects of the desiccation tests on the enzyme gel-banding patterns found in this study. The enzymes examined in this study are listed, along with their abbreviations, enzyme classification number and group affiliations as estimated by Johnson (1974) in Table 4. Estimates of their variability in other Drosophila species are also included. A report on the genetics of some of these systems can be found in Steiner (1975). Gene frequencies were estimated by the gene-counting method. Chi-square tests were done utilizing a computer program written especially for this study by Dr. Peter Garrod, Department of Agricultural Economics, University of Hawaii. The program handles a maximum of 28 phenotypes and six samples.

Three types of Chi-square tests were applied to the data. The first assumed independence between all collections and the distribution of genotypes. The format assumed was that for the R x C table (Snedecor and Cochran, 1969) where each member of a sample was classified by phenotype into the row class and by collection date (season) into a column class. The method assumed independence between the two classes to achieve a nonsignificant Chi-square (χ^2) value.

The second test was made between pairs of collections and the distribution of genotypes. The hypothesis being tested was whether phenotypes comprising the ith collection deviated significantly in frequency from those comprising the jth collection. This test is essentially the 2 x C contingency table test described by Snedecor and Cochran (Ibid., p. 238). Significant Chi-squares in tests one and two indicated that the observed deviations were dependent on the sample. In combination, the tests can be used to determine if there are linear or systematic trends in changes in phenotype frequency if significant heterogeneity exists between the collections.

The third test analyzed the question of Hardy-Weinberg equilibrium within the collections, with a significant Chi-square indicating deviation. It is well recognized that numbers less than 5 may have an effect on determining the asymptotic distribution of the classes when determining the χ^2 statistic (i.e., Alder and Roessler, 1972, p. 236; Lindgren, 1972, p. 297) although Snedecor and Cochran (op. cit.) believe that numbers as low as one can be utilized with reasonable accuracy

TABLE 4. Types of variable enzyme loci as classified by Johnson, 1974.

	Abbreviation	Enzyme Classification No.	Drosophila Heterozygosity*
VARIABLE SUBSTRATE ENZYMES			
Acid phosphatase[a]	ACPH	3.1.3. 1, 2	0.27 (10)
Alkaline phosphatase[a]	APH	3.1.3. 1, 2	0.11 (10)
Peptidases	PEP	---	0.22 (9)
Esterase[a]	EST	---	0.43 (13)
Octanol dehydrogenase[a]	ODH	1.1.1. 1	0.17 (12)
	OVERALL		0.24
REGULATORY ENZYMES			
Adenylate kinase	ADK	2.7.4. 3	0.34 (2)
Alcohol dehydrogenase[a]	ADH	1.1.1. 1	0.28 (12)
Aldehyde oxidase[a]	ALDOX	1.2.1. 3	0.30 (11)
Glyceraldehyde-3-P dehydrogenase[a]	G-3PDH	1.2.1.12	0.18 (2)
Glucose-6-P dehydrogenase	G-6PDH	1.1.1.49	0.11 (8)
Hexokinase[a]	HK	2.7.1. 1	0.08 (6)
Malic enzyme[a]	ME	1.1.1.40	0.08 (12)
Phosphoglucose isomerase	PGI	5.3.1. 9	0.25 (5)
Phosphoglucomutase[a]	PGM	2.7.5. 1	0.20 (9)
Xanthine dehydrogenase[a]	XDH	1.2.3. 2	0.13 (9)
	OVERALL		0.19
NON-REGULATORY ENZYMES			
Aldolase	ALD	4.1.2.13	0.05 (9)
α-Glycerophosphate dehydrogenase[a]	α-GPDH	1.1.1. 8	0.04 (12)
Isocitrate dehydrogenase[a]	IDH	1.1.1.42	0.14 (10)
Fumurase	FUM	4.2.1. 2	0.04 (7)
Malate dehydrogenase[a]	MDH	1.1.1.37	0.02 (13)
6-Phosphogluconate dehydrogenase	6-PGDH	1.1.1.43	0.06 (5)
Lactate dehydrogenase	LDH	1.1.1.28	0.04 (4)
Glutamate oxaloacetate transaminase[a]	GOT	2.6.1. 1	---
Triose P isomerase	TPI	5.3.1. 1	0.04 (4)
	OVERALL		0.06

*pooled estimate (no. of species)

[a]enzyme system studied in this paper

obtained. A possible solution to this problem is the combining of classes having less than, say, 3 individuals. In this study, many phenotypes were encountered in the 4- to 6-allele class loci which were extremely rare. However, because the reality of the biological situation is of importance in this study, and because the season of occurrence of rare phenotypes may be of potential interest, all phenotype classes were maintained as discrete. To compensate for classes lacking individuals in the contingency tables, one degree of freedom was subtracted for each. Classes having expected values of less than one were also listed by the Chi-square program. This enabled recognition of those factors contributing to deviation of the χ^2 statistic toward significance under otherwise homogenous conditions. Because the expected phenotype frequencies are low in some cases, the statistic was calculated on the summed data for each polymorphic locus for each population as well as for each locus within collections. Even then, certain phenotype classes which had low numbers were noticed to contribute to the observed deviations. Because of the low frequency-phenotype class effect, it is suggested that the Hardy-Weinberg statistics be viewed with caution when making judgments about population equilibrium.

A generalized program for data description developed by the laboratory of Dr. M. P. Mi, Department of Genetics, University of Hawaii was utilized to display the gene frequency data. The program compiled the means and standard deviations and also included a supplement test which consisted of an analysis of variance and a multiple-range test with F-value. Description for the technique of analysis of variance of the supplement can be found in Duncan (1955).

RESULTS

(a) Drosophila mimica

The electrophoretic profile with an assumed randomized base revealed the following loci as essentially monomorphic in D. mimica (variability less than 1%): α-GPDH, ADH-1, ADH-2, MDH-1, ME-1, XDH-1, G-3-PDH-1, EST-5, HK-1, APH-1 and GOT-1.

Data on the gene frequencies for the ODH-1, LAP-2, and ALDOX-1 loci is presented in Tables 5-7. At the ODH-1 locus (Table 5), technical difficulties in stain technique prevented adequate numbers to be sampled although the allele frequencies obtained fit very close to those of Rockwood (1969). For the LAP-2 locus (Table 6), Rockwood (1971) found only two alleles. This study exposes four with the fastest migrating allele ($LAP\text{-}2^2$) occurring at 1% frequency in the December collection only. These differences are probably due to the types of gel starch used

TABLE 5. Gene frequencies at the 2 allele Octanol Dehydrogenase-1 (ODH-1) locus in two D. mimica populations.

Population	Collection	N	4	5	χ^{2A}	χ^{2B}
Kipuka Ki	JUL	13	.808 ± .10	.192 ± .10	NS	
	JAN	41	.841 ± .06	.159 ± .06	NS	
	MAR	6	1.000	---	NS	
	JUN	14	.893 ± .08	.107 ± .08	NS	
	SEP	6	.917 ± .10	.083 ± .10	NS	
	TOTAL	80	.863 ± .04	.138 ± .04	NS	NS
Kipuka Puaulu	JUL	22	.818 ± .06	.182 ± .06	NS	
	JAN	39	.769 ± .06	.231 ± .06	NS	
	MAR	6	.500 ± .20	.500 ± .20	NS	
	JUN	10	.800 ± .10	.200 ± .10	NS	
	SEP	5	.700 ± .21	.300 ± .21	NS	
	TOTAL	82	.762 ± .05	.238 ± .05	NS	NS

A Hardy-Weinberg test

B Contingency test

NS Not significant

TABLE 6. Gene frequencies at the 4 allele Leucine aminopeptidase (LAP-2) locus in two D. mimica populations.

Population	Collection	N	2	3	4	5	χ^{2A}	χ^{2B}
Kipuka Ki	JUN	134	---	.022 ± .01	.862 ± .03	.116 ± .03	17.21(3)***	
	SEP	85	---	.012 ± .01	.971 ± .02	.018 ± .08	37.12(3)***	
	DEC	135	.015 ± .01	.359 ± .04	.574 ± .04	.052 ± .02	24.98(6)***	
	TOTAL	354	.006 ± .004	.146 ± .02	.773 ± .02	.075 ± .01	116.95(6)***	179.2(37)***
Kipuka Puaulu	JUL	22	---	.023 ± .03	.500 ± .10	.477 ± .10	NS	
	MAR	3	---	---	1.000	---	---	
	JUN	149	---	.013 ± .01	.943 ± .02	.044 ± .01	11.49(3)***	
	SEP	170	---	.018 ± .01	.950 ± .02	.032 ± .01	NS	
	DEC	127	.012 ± .01	.453 ± .05	.524 ± .05	.012 ± .01	NS	
	TOTAL	471	.003 ± .002	.134 ± .01	.812 ± .02	.051 ± .02	149.38(6)***	363.9(37)***

A Hardy-Weinberg test (df in parenthesis)

B Contingency test (df in parenthesis)

NS Not significant

*** P <0.001

in the analysis. For the ALDOX locus (Table 7) limited collections were made. In addition, there appears to be some effect due to differences in gel starch lot here as well. All the above considerations have resulted in inconsistencies at these three loci. Thus we suggest that data for these loci be viewed with some reservation, although their inclusion in the number of loci polymorphic within the species appears valid. The data for these loci is presented to demonstrate that extensive and complex polymorphism exists for the ALDOX and LAP-2 loci. It is interesting to note, for example, that heterogeneity in phenotype frequencies exists between collections for the Leucine Aminopeptidase locus even on gels with good resolving power (DEC collection, contingency χ^2 = 179.2 and 363.9). The extent this phenomenon is independent of low-frequency phenotype classes and is a true reflection of events associated with this locus must form the nucleus of a future study.

Gene frequency data for polymorphic gene systems which were consistently scorable are presented in Tables 8 through 21. Loci comprising this group include ACPH-1, APH-3, EST-2, HK-3, IDH-1, LAP-1 and PGM-1. Of these, only ACPH-1 and APH-3 have been reported on in detail in the literature (Rockwood, 1969).

Examination of Table 8 reveals evidence favoring Rockwood's (1969) earlier hypothesis of successional changes at the ACPH locus in Kipuka Ki. The homozygote 33 type as well as allele frequencies for ACPH-1^3 appear to be cyclical in nature. The picture is not as clear cut for Kipuka Puaulu, a fact which was also found by Rockwood.

At the APH-3 locus, Rockwood found the frequencies of the six alleles to be stable over the time span of her study. She advanced Levene's (1953) hypothesis that multiple ecological niches available to this species could be the factor supporting this system. The model assumes a randomly mating population depositing zygotes at random into each niche with differential mortality within the niche. The data of Table 9 also supports Rockwood's findings, finding gene frequencies fairly consistent over time. There are no consistent patterns of change at any one allele although visual inspection of both the Rockwood data and the data of this study suggests that allele APH-3^4 (allele 3 in Rockwood) is higher in frequency during winter months.

A pairwise test to detect heterogeneity between sample phenotype class frequencies proved negative although the contingency χ^2 for Kipuka Puaulu is significant. Deviations detected by the Hardy-Weinberg test are not due to any excess or deficiency of homozygotes to heterozygotes within collections and are probably a reflection of the number of small phenotype classes present. The homogeneity χ^2 and

TABLE 7. Gene frequencies at the 5 allele Aldehyde Oxidase-1 (ALDOX-1) locus in two D. mimica populations.

Population	Collection	N	2	3	4	5	6	χ^{2A}	χ^{2B}
Kipuka Ki	JUN	71	.141 ± .04	.042 ± .02	.627 ± .06	.141 ± .04	.049 ± .02	39.68(10)***	
	DEC	70	.021 ± .02	.393 ± .06	.443 ± .06	.142 ± .04	---	46.30(6)***	
	TOTAL	141	.096 ± .03	.202 ± .04	.535 ± .04	.142 ± .03	.025 ± .01	72.14(10)***	65.3(23)***
Kipuka Puaulu	JUN	87	.063 ± .03	.092 ± .03	.649 ± .05	.115 ± .03	.085 ± .03	54.87(10)***	
	DEC	32	.125 ± .03	.359 ± .08	.375 ± .09	.141 ± .03	---	NS	
	TOTAL	119	.080 ± .02	.164 ± .03	.576 ± .04	.122 ± .03	.059 ± .01	48.42(10)***	38.7(20)***

A Hardy-Weinberg Test, $P < 0.001$ (df in parenthesis)

B Contingency test, $P < 0.001$ (df in parenthesis)

NS Not significant

*** $P < 0.001$

TABLE 8. Gene frequencies and phenotype observations at the Acid phosphatase-1 (ACPH-1) locus in the two *D. mimica* populations.

This study		Alleles					Phenotypes					
Population	Collection	3	4	5	χ^{2A}	χ^{2B}	33	34	35	44	45	55
Kipuka Ki	JUL	.350 ± .05	.618 ± .06	.032 ± .02	NS		13	34		36	5	
	JAN	.300 ± .03	.664 ± .05	.036 ± .02	NS		18	50	1	68	9	1
	MAR	.289 ± .03	.658 ± .07	.053 ± .02	NS		10	32	2	38	6	1
	JUN	.245 ± .04	.734 ± .04	.022 ± .01	NS		7	54	3	72	3	
	SEP	.307 ± .05	.641 ± .05	.052 ± .02	NS		7	44	1	36	7	1
	DEC	.330 ± .03	.628 ± .04	.043 ± .01	NS		24	91	8	89	11	
	Total	.303 ± .01	.657 ± .02	.039 ± .006	NS	NS	89	305	15	339	41	3
Kipuka Puaulu	JUL	.290 ± .04	.680 ± .05	.030 ± .02	NS		2	25		20	2	
	JAN	.322 ± .04	.616 ± .04	.062 ± .02	13.10 (3)***		20	37		47	14	
	MAR	.394 ± .05	.553 ± .05	.053 ± .02	NS		18	51	2	33	8	1
	JUN	.284 ± .04	.671 ± .04	.045 ± .02	NS		12	56	3	65	10	
	SEP	.318 ± .03	.647 ± .03	.035 ± .03	NS		24	65	4	83	7	1
	DEC	.358 ± .04	.602 ± .04	.040 ± .02	NS		17	62	2	47	9	
	Total	.329 ± .01	.626 ± .02	.045 ± .01	8.66 (3)*	NS	93	296	11	295	50	2

A Hardy-Weinberg Test (df in parenthesis)

B contingency Test

NS Not significant

* $P < 0.05$

*** $P < 0.001$

TABLE 9. Gene frequencies and phenotype observations at the alkaline phosphatase (APH-3) locus in two D. mimica populations.

Population	Collection	Alleles 2	3	4	5	6	7	χ^{2A}
Kipuka Ki	JUL	.026 ± .02	.140 ± .05	.351 ± .06	.298 ± .06	.114 ± .05	.071 ± .03	NS
	JAN	.033 ± .02	.205 ± .04	.319 ± .05	.324 ± .05	.081 ± .03	.038 ± .02	26.06(15)*
	MAR	.043 ± .04	.171 ± .06	.357 ± .08	.229 ± .07	.157 ± .06	.043 ± .04	NS
	JUN	.064 ± .04	.192 ± .06	.397 ± .08	.218 ± .07	.064 ± .04	.065 ± .04	32.88(15)**
	SEP	.065 ± .05	.174 ± .08	.348 ± .10	.261 ± .09	.087 ± .06	.065 ± .04	NS
	DEC	.011 ± .02	.200 ± .06	.344 ± .07	.300 ± .07	.111 ± .05	.034 ± .03	NS
	Total	.036 ± .01	.184 ± .02	.344 ± .03	.287 ± .03	.098 ± .02	.049 ± .01	NS
Kipuka Puaulu	JUL	---	.066 ± .03	.401 ± .06	.290 ± .05	.132 ± .04	.111 ± .04	20.85(10)*
	JAN	029 ± .02	.188 ± .05	.348 ± .06	.297 ± .05	.080 ± .03	.058 ± .03	NS
	MAR	.038 ± .03	.132 ± .05	.246 ± .06	.377 ± .07	.132 ± .05	.057 ± .03	NS
	JUN	.094 ± .04	.219 ± .06	.313 ± .07	.240 ± .06	.125 ± .04	.009 ± .01	27.80(15)*
	SEP	.030 ± .02	.089 ± .03	.400 ± .05	.369 ± .05	.077 ± .03	.035 ± .02	NS
	DEC	---	.075 ± .06	.325 ± .11	.350 ± .11	.175 ± .08	.075 ± .06	19.90(10)*
	Total	.031 ± .01	.127 ± .02	.353 ± .03	.320 ± .03	.110 ± .02	.056 ± .01	49.47(15)**

A Hardy-Weinberg Test (df in parenthesis)

B Contingency Test

NS Not significant

* $P < 0.05$

*** $P < 0.01$

(continued)

TABLE 9. (concluded) Gene frequencies and phenotype observations at the (APH-3) locus, D. mimica.

Phenotypes																			
22	23	24	25	26	27	33	34	35	36	37	44	45	46	47	55	56	57	66	67	77
		1	1	1			6	7	1		6	6	4	2	5	5	2		2	1
1	1	1	1			4	13	10		3	8	26	2	3	8	6	2	2		
		1	1			1	5	5			4	5	4	1		1	1	1	1	
	2	1	1		1	3	6	1			9	2	2	2	3	1			2	
	1		1		1	2		2	1		4	4	2	2	2	1				
		1				2	6	5	1	2	4	11	5		4	3			1	
1	4	5	5	1	2	12	36	30	3	5	35	54	19	10	22	17	5	3	6	1
							4	5		1	8	21	12	5	6	5	1	1	1	4
	1	1	2			3	9	5	2	2	10	11	5	2	9	2	3	1		
	1		3			2		4	2	3	7	8	4	2	8	8	1			
2	3	1		1		2	10	2	2		7	5			5	5	1	2		
1	2	1	1		1		7	8			12	25	7	3	11	4	2	1		
								1	1	1	1	9	2		1	2		1		1
3	7	2	6	1	1	7	30	25	7	7	45	79	30	12	40	26	8	6	1	5

the Hardy-Weinberg total χ^2 deviations which are highly significant are, however, due in part to a slight excess of heterozygotes across all collections.

An analysis of variance of the allele frequencies revealed the existence of heterogeneity at only 2 alleles, APH-3^2 and APH-3^3 (Table 10). Examination of the data indicates the basis for the heterogeneity lies in the observation that allele 3 is higher in frequency in summer months while allele 2 is lower with the situation reversed for winter months. Inspection of Rockwood's data, however, suggests that this observation does not hold true as to month of occurrence, implying some environmental parameter independent of season may be involved. Because the pairwise contingency tests showed no significant differences in phenotype frequencies, the above trend was not investigated further.

Table 11 displays the gene frequencies and phenotype counts for 6 collections at the LAP-1 locus. Strong deviations from Hardy-Weinberg expectations are observed for all samples except the January and September, 1973 collections in Kipuka Ki and the September and December, 1973 collections in Kipuka Puaulu. Again the deviations from the observed expectations are due in part to the occurrence of low-frequency phenotype classes (i.e., phenotype classes 22, 25, 36, 56 and 66). A large part of the deviation is due, however, to genetic events at this locus. Inspection of the data shows that certain phenotype classes fluctuate in frequency between collections in an apparently random manner. This fluctuation can be observed in the allele frequencies as well. A Duncan multiple Range Test with analysis of variance supports this contention by demonstrating significant heterogeneity between collections for the alleles LAP-1^3, LAP-1^5 and LAP-1^6 (Table 12).

Another factor contributing to the deviations from Hardy-Weinberg equilibrium at the LAP-1 locus is a noticeable lack of heterozygotes (Table 13). The deficiency is significant for the January 1973 collection in Kipuka Ki, and highly significant when summed over all collections for both populations. For the Kipuka Puaulu population, the July, 1972, March, 1973 and June, 1973 collections deviate quite significantly. The lack of heterozygotes is not expected under either the assumption of random-mating in the population or at such a highly polymorphic locus (range $\bar{h}$ = 0.574 - 0.661, Table 14).

Similar observations are seen for the EST-2 locus in D. mimica (Table 15). Pairwise tests for homogeneity indicate that phenotype frequencies in Kipuka Puaulu collections differ significantly from each other (Table 16). Kipuka Ki populations do not display this tendency. In addition, the Hardy-Weinberg test for genetic equilibrium shows each collection deviating significantly from the expected.

TABLE 10. Analysis of variance in allele frequencies observed between collections at the APH-3 locus in *D. mimica* (populations pooled).

Allele	Source	df	Sum Squares	Mean Squares	F
2	Total Collections	653	12.2599		
	Between Collections	11	0.3737	0.0339	1.84*
	Within Collections	642	11.8862	0.0185	
3	Total Collections	653	45.3061		
	Between Collections	11	1.8297	0.1663	2.46*
	Within Collections	642	43.4765	0.0677	

* $P < 0.05$

** $P < 0.01$

TABLE 11. Gene frequencies and phenotype observations at the leucine aminopeptidase-1 (LAP-1) locus in two D. mimica populations.

Population	Collection	Alleles 2	3	4	5	6	χ^{2A}	χ^{2B}
Kipuka Ki	JUL	---	.458 ± .08	.444 ± .08	.097 ± .05	---	12.86 (3)***	
	JAN	.023 ± .02	.284 ± .05	.381 ± .05	.313 ± .05		60.69 (6)***	
	MAR	.022 ± .02	.289 ± .07	.522 ± .07	.167 ± .06	---	NS	
	JUN	.027 ± .02	.142 ± .04	.311 ± .05	.331 ± .05	.088 ± .03	114.89 (10)***	
	SEP	---	.394 ± .09	.288 ± .09	.318 ± .09	---	12.48 (3)*	
	DEC	.044 ± .03	.395 ± .06	.518 ± .07	.044 ± .03	---	19.03 (6)***	
	Total	.018 ± .01	.281 ± .03	.453 ± .03	.226 ± .02	.023 ± .01	299.91 (10)***	NS
Kipuka Puaulu	JUL	---	.410 ± .06	.373 ± .06	.216 ± .05	---	46.27 (3)***	
	JAN	.007 ± .01	.204 ± .05	.401 ± .06	.380 ± .06	.007 ± .005	28.78 (10)***	
	MAR	.032 ± .02	.186 ± .04	.474 ± .06	.308 ± .05	---	46.81 (6)***	
	JUN	.012 ± .01	.191 ± .04	.429 ± .05	.310 ± .05	.060 ± .03	20.90 (10)*	
	SEP	.005 ± .01	.279 ± .04	.394 ± .05	.317 ± .05	.005 ± .01	NS	
	DEC	.021 ± .02	.362 ± .07	.564 ± .07	.053 ± .03	---	NS	
	Total	.015 ± .01	.267 ± .02	.434 ± .02	.270 ± .02	.014 ± .01	127.26 (10)***	***

A Hardy-Weinberg Test (df in parenthesis)

B contingency Test

NS Not significant

* $P < 0.05$

*** $P < 0.001$

(continued)

TABLE 11. (concluded) Gene frequencies and phenotype observations at the LAP-1 locus in D. mimica.

Phenotypes[b]													
22	23	24	25	33	34	35	36	44	45	46	55	56	66
				4	10	2		10	1		2		
1	2			12	8	7		22	9		12		
	2			6	11	1		13	8		3		
2				5	5	5	1	19	17	1	12	3	4
				9	2	6		6	5		5		
	2			9	13	1		19	2		1		
3	6			45	49	22	1	79	42	1	35	3	4
				21	8	4		17	5		10		
		1		5	12	7		17	7	1	20		
1	2		1	10	7	2		23	19		13		
		1	1	8	7	6	3	20	20	4	12	1	1
	1			12	17	16		20	25		12	1	
	1	4		10	24			14	3		1		
1	4	6	2	66	75	35	3	111	79	5	68	2	1

b No observations for phenotype 26

TABLE 12. Analysis of variance of allele frequencies at the LAP-1 locus in *D. mimica*.

Allele	Source	df	Sum Square	Mean Square	F
3	Total	783	107.8251		
	Between	11	6.9653	0.6332	4.85*
	Within	772	100.8597	0.1306	
5	Total	783	102.9374		
	Between	11	8.6255	0.7841	6.42**
	Within	772	94.3118	0.1222	
6	Total	783	8.5507		
	Between	11	0.6752	0.0614	6.02**
	Within	772	7.8755	0.0102	

** $P < 0.01$

TABLE 13. Deviations of observed numbers of heterozygotes from the expected in natural populations of D. mimica at 2 gene loci.

Locus	Population	Collection	obs	exp	χ^{2A}
LAP-1	Kipuka Ki	JUL	13	21	NS
		JAN	26	43	6.33*
		MAR	22	27	NS
		JUN	32	40	NS
		SEP	13	22	NS
		DEC	18	25	NS
		Total	124	178	16.08***
	Kipuka Puaulu	JUL	15	37	12.49***
		JAN	28	39	NS
		MAR	31	54	9.38**
		JUN	22	57	20.88***
		SEP	57	66	NS
		DEC	27	30	NS
		Total	180	283	37.12
EST-2	Kipuka Ki	JUL	39	48	NS
		JAN	49	69	5.51*
		MAR	43	55	NS
		JUN	35	52	5.24*
		SEP	23	34	NS
		DEC	76	88	NS
		Total	265	346	18.73***
	Kipuka Puaulu	JUL	23	27	NS
		JAN	57	58	NS
		MAR	54	72	4.25*
		JUN	39	54	NS
		SEP	39	73	15.37***
		DEC	84	72	NS
		Total	296	356	9.94**

A Corrected with Yates Correction Factor for 1 df.

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

TABLE 14. Population and locus heterozygosity values for D. mimica.[a]

Population	Collection	ACPH-1	APH-3	EST-2	HK-3	IDH-1	LAP-1	PGM-1	$\bar{H}$
Kipuka Ki	JUL	0.495	0.748	0.624	0.397	0.263	0.582	0.249	0.198
	JAN	0.468	0.743	0.572	---[b]	0.124	0.618	0.182	0.169
	MAR	0.481	0.766	0.664	---[b]	0.239	0.616	0.065	0.177
	JUN	0.403	0.745	0.675	0.311	0.273	0.574	0.311	0.194
	SEP	0.493	0.766	0.613	0.368	0.186	0.661	0.411	0.206
	DEC	0.496	0.738	0.650	0.289	0.135	0.575	0.263	0.185
Average	$\bar{h}$	0.473	0.745	0.651	0.340	0.216	0.666	0.203	0.194
Kipuka Puaulu	JUL	0.452	0.721	0.595	0.357	0.282	0.644	0.134	0.187
	JAN	0.513	0.745	0.567	---[b]	0.229	0.652	0.132	0.177
	MAR	0.537	0.659	0.670	---[b]	0.270	0.645	0.189	0.185
	JUN	0.467	0.772	0.654	0.165	0.226	0.682	0.220	0.187
	SEP	0.478	0.688	0.629	0.296	0.226	0.667	0.060	0.179
	DEC	0.508	0.732	0.676	0.165	0.135	0.517	0.325	0.182
Average	$\bar{h}$	0.498	0.734	0.660	0.269	0.223	0.666	0.158	0.189

a See Table 3 for description of $\bar{h}$ and $\bar{H}$.

b Not analyzed due to technical difficulty.

TABLE 15. Gene frequencies and phenotype observations at the Esterase-2 (EST-2) locus in two D. mimica populations.

Population	Collection	Alleles 2	3	4	5	6	7	χ^{2A}	χ^{2B}
Kipuka Ki	JUL	.012 ± .01	.133 ± .04	.524 ± .05	.283 ± .04	.042 ± .02	.006 ± .005	31.88 (15)***	
	JAN	---	.157 ± .03	.589 ± .04	.238 ± .03	.016 ± .01	---	23.11 (6)***	
	MAR	.023 ± .01	.376 ± .05	.410 ± .05	.157 ± .04	.028 ± .02	.006 ± .005	72.79 (15)***	
	JUN	.039 ± .02	.175 ± .04	.474 ± .06	.253 ± .05	.058 ± .03	---	28.37 (10)***	
	SEP	---	.164 ± .05	.546 ± .07	.200 ± .05	.082 ± .04	---	24.75 (10)***	
	DEC	.078 ± .02	.310 ± .04	.481 ± .04	.127 ± .03	.004 ± .003	---	36.41 (10)***	
	Total	.029 ± .001	.227 ± .02	.504 ± .02	.203 ± .02	.032 ± .01	.005 ± .003	185.98 (15)***	**
Kipuka Puaulu	JUL	---	.094 ± .04	.573 ± .07	.250 ± .06	.083 ± .04	---	15.87 (6)***	
	JAN	.019 ± .01	.170 ± .04	.607 ± .05	.185 ± .04	.019 ± .01	---	60.66 (10)***	
	MAR	.037 ± .02	.208 ± .04	.444 ± .05	.296 ± .04	.009 ± .008	.006 ± .005	32.6 (15)***	
	JUN	.085 ± .03	.122 ± .04	.518 ± .06	.226 ± .05	.043 ± .02	.006 ± .003	32.6 (15)**	
	SEP	.026 ± .01	.125 ± .03	.535 ± .05	.254 ± .04	.043 ± .02	.017 ± .01	150.23 (15)***	
	DEC	.094 ± .03	.401 ± .05	.373 ± .05	.123 ± .03	.009 ± .001	---	18.24 (10)*	
	Total	.047 ± .01	.198 ± .02	.500 ± .02	.220 ± .02	.029 ± .01	.006 ± .003	233.56 (15)***	***

A Hardy-Weinberg Test (df in parenthesis)

B contingency test

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

(continued)

TABLE 15. (Concluded) Gene frequencies and phenotype observations at the EST-2 locus in D. mimica.

Phenotypes																		
22	23	24	25	26	33	34	35	36	44	45	46	47	55	56	57	66	67	77
	1	1			3	10		3	24	22		1	10	1		1		
					7	16	6	1	51	23	1		13	2				
	2	2			17	25	3		15	8	2		7			1	1	
	1	4	1		7	4	7	1	25	11	4		9	2		1		
					5	5	3		21	9	3	1	4	2		2		
5	8	3			12	40	10	1	36	14			5					
5	12	10	1		51	100	29	6	172	87	10	2	48	7		5	1	
					1	4	1	2	16	14	2		4			1		
1	1	1			1	29	3		36	21	2		7			1		
1	2	4			9	19	6		26	20		1	18	2				
2	4	5	1		3	6	3	1	28	14	4		9		1	1		
2		1	1		7	12	2	1	47	16	1		18	3	1	2	1	1
1	11	6		1	9	41	15		11	9	1		1					
7	18	17	2	1	30	111	30	4	164	94	10	1	57	5	2	5	1	1

TABLE 16. Chi-Square statistics for a pairwise test of homogeneity in phenotype frequencies at the EST-2 locus in two populations of *D. mimica*.

DEC	JUL	JAN	MAR	JUN	SEP
Kipuka Ki	42.15	40.33	20.66	40.96	37.56
Kipuka Puaulu	63.23***	53.26**	49.00*	60.37***	91.21***

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

Low-frequency phenotype classes contributing to this phenomena include phenotypes 25, 47, 57, 67 and 77 (Table 15). Other factors contributing to this are a series of events similar to those observed at the LAP-1 locus. In Kipuka Ki, for example, the most common allele has a frequency range from 0.41 - 0.58, the variance between collections being significantly different from homogeneity ($P < 0.01$). Table 17 indicates this is typical for all alleles at this locus. The observation is reflected in the random fluctuation of phenotype frequencies over time.

Another factor contributing to the large disequilibrium in gene frequencies (assuming panmixia, no migration and no selection) is the deficiency of heterozygotes at this locus (Table 13). The deficiency is significant for the January and June Kipuka Ki collections and the March and September Kipuka Puaulu collections, and highly significant when summed across collections for each population ($P < 0.001$). The enzyme is thought to act on external substrates as well as having possible internal metabolic functions (Johnson, 1974). If Levene's (1953) hypothesis concerning differential niche utilization by different phenotypes is applied to this case, such an observation would be consistent with non-random mating taking place within the niche. Rockwood (1969) discussed this possibility concerning the high frequencies attained by the alleles at the APH-3 locus (see Table 9).

At the Hexokinase-3 locus, both populations are in Hardy-Weinberg equilibrium, even though phenotype classes 33 and 35 are in low frequency (Table 18). A test for homogeneity verifies that the populations are stable at this locus over time, although the multiple Range Test for heterogeneity in the variances in allele frequencies proves significant for alleles 3 and 5 (Table 19). Inspection of the allele frequencies suggests the reason for this is due primarily to the unusually high occurrence of $HK\text{-}3^{3}$ in the Kipuka Puaulu July 1972 sample and the lack of allele $HK\text{-}3^{5}$ in the July 1972 and December 1973 samples. The highest frequency of $HK\text{-}3^{3}$ is followed by the lowest observed frequency in the June 1973 sample. It is difficult to determine if this decline was systematic or erratic in course due to a loss of the January 1973 and March 1973 samples because of technical difficulties. The change is consistent in both populations, however.

The results of the analysis for the IDH-1 locus is given in Table 20. Only the January 1973 collection from Kipuka Puaulu deviates significantly from Hardy-Weinberg equilibrium ($P < 0.05$). The pooled data for that population also shows a significant deviation. These deviations are not due to low-frequency phenotype classes as none are present in the Kipuka Puaulu sample. Table 20 suggests a more likely explanation may involve selective factors. It appears that the allele $IDH\text{-}1^{5}$

TABLE 17. Analysis of the variance observed in EST-2 allele frequencies between collections in *D. mimica* (populations pooled).

Allele	Source	df	Sum Squares	Mean Squares	F
2	Total	1124	25.6444		
	Between	11	1.1966	0.1088	4.95**
	Within	1113	24.4478	0.0220	
3	Total	1124	109.7639		
	Between	11	10.9939	0.9994	11.26**
	Within	1113	98.7691	0.0887	
4	Total	1124	168.7389		
	Between	11	5.5923	0.5084	3.47**
	Within	1113	163.1467	0.1465	
5	Total	1124	122.1879		
	Between	11	3.8636	0.3512	3.30**
	Within	1113	118.3243	0.1063	
6	Total	1124	21.4724		
	Between	11	0.6113	0.0556	2.97**
	Within	1113	20.8611	0.0187	

** $P < 0.01$

TABLE 18. Gene frequencies and phenotype observations at the Hexokinase-3 locus (HK-3) in two *D. mimica* populations.

Population	Collection	Alleles 3	Alleles 4	Alleles 5	χ^{2A}	χ^{2B}	Phenotypes 33	Phenotypes 34	Phenotypes 35	Phenotypes 44	Phenotypes 45	Phenotypes 55
Kipuka Ki	JUL	.121 ± .06	.759 ± .08	.121 ± .06	NS		1	4		14	7	
	JUN	.077 ± .04	.821 ± .06	.103 ± .05	NS			5	1	27	5	1
	SEP	.066 ± .03	.776 ± .04	.158 ± .04	NS		1	11		59	23	4
	DEC	.069 ± .03	.836 ± .04	.096 ± .04	NS		1	7	1	52	11	1
	Total	.075 ± .01	.799 ± .05	.126 ± .02	NS	NS	3	27	2	152	46	6
Kipuka PUaulu	JUL	.233 ± .10	.767 ± .10	---	NS			7		8		--
	JUN	.022 ± .02	.911 ± .04	.067 ± .04	NS			2		37	6	--
	SEP	.091 ± .03	.831 ± .04	.079 ± .03	NS		2	16	3	89	17	--
	DEC	.091 ± .09	.909 ± .09	---	NS			2		9		--
	Total	.086 ± .02	.849 ± .03	.066 ± .02	NS	NS	2	27	3	143	23	--

A Hardy-Weinberg Test

B Contingency Test

NS Not significant

TABLE 19. Analysis of the variance observed in HK-3 allele frequencies between collections in *D. mimica* (populations pooled).

Allele	Source	df	Sum Square	Mean Square	F
3	Total	436	17.1968		
	Between	7	0.5947	0.0849	2.20*
	Within	429	16.6021	0.0387	
5	Total	436	20.2689		
	Between	7	0.7117	0.1017	2.23*
	Within	429	19.5572	0.0456	

* $P < 0.05$

TABLE 20. Gene frequencies at the isocitrate dehydrogenase-1 (IDH-1) locus in two D. mimica populations.

		Alleles					Phenotypes[2]			
Population	Collection	3	4	5	χ^{2A}	χ^{2B}	34	44	45	55
Kipuka Ki	JUL	.007 ± .003	.847 ± .04	.147 ± .04	NS		1	49	15	1
	JAN		.855 ± .04	.145 ± .04	NS			73	16	10
	MAR		.861 ± .06	.139 ± .06	NS			26	10	
	JUN		.837 ± .03	.163 ± .03	NS			96	39	3
	SEP		.896 ± .06	.104 ± .06	NS			20	3	1
	DEC		.927 ± .02	.073 ± .02	NS			171	27	1
	Total	.001 ± .001	.877 ± .01	.122 ± .01	NS	*	1	435	110	16
Kipuka Puaulu	JUL		.830 ± .04	.170 ± .04	NS			66	24	3
	JAN		.868 ± .04	.132 ± .04	5.37 (1)*			68	15	4
	MAR	.839 ± .05	.161 ± .05	NS				41	12	3
	JUN		.870 ± .03	.130 ± .03	NS			113	35	2
	SEP		.870 ± .04	.130 ± .04	NS			60	14	3
	DEC		.927 ± .02	.073 ± .02	NS			101	15	1
	Total		.872 ± .01	.128 ± .01	7.95 (1)**	NS	449	115	16	

a No observations for phenotypes 33, 35

A Hardy-Weinberg Test (df in parenthesis)

B Contingency Test

NS Not significant

* $P < 0.05$

** $P < 0.01$

is declining in frequency over the course of this study while allele IDH-1^4 is increasing. An analysis of variance of the allele frequencies reveals that there is indeed heterogeneity between collections ($F = 2.25$, 11 df, $P < 0.05$ for IDH-1^4; $F = 2.22$, 11 df, $P < 0.05$ for IDH-1^5).

At the PGM-1 locus, a contingency test for homogeneity reveals no significant differences between collections with respect to phenotype frequencies (Table 21). However, several of the samples are not in Hardy-Weinberg equilibrium. These include the December 1973 Kipuka Ki collection and the March, September and December (1973) collections. In addition, the summed data for each sample is not at equilibrium. Low-frequency phenotype classes which bias the Chi-square upwards include phenotypes 23, 24, 34 and 55. In addition, part of the high values can be attributed to significant heterogeneity between collections in allele frequencies ($F = 7.81$, 11 df, $P < 0.01$ for PGM-1^4; $F = 8.23$, 11 df, $P < 0.01$ for PGM-1^5).

Table 14 reveals the extent of variation observed in the two D. mimica populations. General population heterozygosity ($\bar{H}$) summed over collections is slightly higher in Kipuka Ki than Kipuka Puaulu. Within-locus levels of variability apparently vary between the collections when comparing populations, however. For example, ACPH-1 is generally lower in variability in Kipuka Ki than Kipuka Puaulu, exceptions occurring for the July 1972 and September 1973 collections. It is interesting to note that ten of 21 loci had significant levels of heterozygosity present (= 47.6% of loci sampled were polymorphic).

(b) Drosophila engyochracea

In D. engyochracea a total of 20 gene-enzyme loci were examined for variability. Of these, no variability were found at the following loci: α-GPDH, G-3-PDH, IDH, HK-1, EST-5, ACPH, GOT-1, LAP-1 and XDH. Variability in the form of rare alleles was observed at 5 genes including: MDH-1, ADH-1, ADH-2, ME and HK-3. These loci were considered essentially monomorphic and not investigated further. All the above loci were used in the determination of percent of polymorphic loci in D. engyochracea as well as the calculation of population heterozygosity estimates. No activity was detected for APH on the starch gels.

Six loci were found to be polymorphic with codominant gene systems present. These include PGM-1, EST-2, ODH-1, LAP-2, ALDOX-1 and EST-3. Although the considerations given to the ALDOX locus in D. mimica apply here as well, a genetic analysis of the ALDOX-1 is given, and it is included in estimates concerned with calculating population heterozygosity, locus heterozygosity and percent of polymorphic loci. This is because electrophoretic separation of the alleles at this locus is

TABLE 21. Gene frequencies and phenotype observations at the phosphoglucomutase (PGM-1) locus in two *D. mimica* populations.

Population	Collection	Alleles 2	3	4	5	6	χ^{2A}	χ^{2B}
Kipuka Ki	JUL	.020 ± .01	.066 ± .08	.863 ± .03	.051 ± .01	---	NS	
	JAN		.091 ± .04	.900 ± .04	.009 ± .01	---	NS	
	MAR		.013 ± .01	.967 ± .02	.030 ± .02	---	NS	
	JUN	.004 ± .01	.115 ± .03	.820 ± .03	.061 ± .02	---	NS	
	SEP		.005 ± .01	.979 ± .01	.016 ± .01	---	NS	
	DEC		.124 ± .03	.844 ± .04	.028 ± .02	---	16.22 (3)***	
	Total	.005 ± .001	.071 ± .01	.888 ± .01	.035 ± .01	---	26.26 (6)***	NS
Kipuka Puaulu	JUL	.005 ± .01	.052 ± .02	.930 ± .03	.014 ± .01	---	NS	
	JAN		.035 ± .03	.931 ± .03	.035 ± .02	---	NS	
	MAR		.020 ± .02	.953 ± .03	.027 ± .02	---	17.79 (3)***	
	JUN		.074 ± .02	.879 ± .03	.047 ± .02	---	NS	
	SEP		.005 ± .01	.969 ± .01	.026 ± .01	---	46.61 (3)***	
	DEC	.005 ± .01	.149 ± .04	.798 ± .04	.037 ± .02	.005 ± .005	16.51 (6)***	
	Total	.001 ± .001	.052 ± .01	.915 ± .04	.031 ± .01	.001	63.36 (6)***	NS

A Hardy-Weinberg Test (df in parenthesis)

B Contingency Test

NS Not significant

*** $P < 0.001$

(continued)

TABLE 21. (Concluded) Gene frequencies and phenotype observations at the PGM-1 locus, D. mimica.

		Phenotypes[b]							
Population	Collection	23	24	33	34	35	44	45	55
Kipuka Ki	JUL		5	2	12	1	86	10	1
	JAN			1	8		45		
	MAR				1		68	2	
	JUN	1		4	22	1	96	16	
	SEP				1		92	3	
	DEC			2	53	1	82	3	1
	Total	1	5	9	97	3	469	34	2
Kipuka Puaulu	JUL		1	1	8	1	93	2	
	JAN				4		50	4	
	MAR				3		69	2	1
	JUN			3	15	1	117	13	
	SEP				1	1	182	5	2
	DEC		1	6	15	1	65	4	1
	Total		2	10	46	4	576	30	4

b No observations for phenotypes 22 and 25.

better in D. engyochracea than in D. mimica. The genetic data for this enzyme are found in Table 22.

A contingency Chi-square test for homogeneity indicates the collections of both populations are significantly different in phenotype frequencies. However, for Kipuka Puaulu, a pairwise contingency test demonstrated no significant differences between any two populations. The same test applied to the Kipuka Ki populations indicated that the June collection differed significantly from the March collection ($P < 0.05$) and the September sample ($P < 0.01$) while the September sample differed significantly from the March collection ($P < 0.001$). These differences are due to the fluctuations in phenotype classes 33 and 44 with 33 increasing over time.

Examination of allele frequencies shows that allele ALDOX-1^3 is increasing during this study. This is due primarily to the above observation on the increase in phenotype 33. The increase is accompanied by a decay in allele ALDOX-1^4. It should be noted, however, that collections for both populations are essentially in Hardy-Weinberg equilibrium except for the December 1973 and March 1974 samples. These large estimates are due in part to low expectations in phenotype classes 22, 25, 35 and 55.

Significant heterogeneity ($P < 0.01$) is observed in the variance of the frequencies of alleles ALDOX-1^2, ALDOX-1^3, ALDOX-1^4 and ALDOX-1^5 but not ALDOX-1^6 (Table 23). In alleles ALDOX-1^2 and ALDOX-1^5, the variance is due to the lack of the allele being detected (i.e., the March Kipuka Puaulu collection for ALDOX-1^5) or the allele being present in extremely low frequency in some collections while it is high enough in other collections to result in an inflated variance estimate. For these alleles, estimates are dependent on sample size to a strong degree; the point holds true for any allele occurring at less than 5 - 10% in frequency. The significant variance at the ALDOX-1^3 and ALDOX-1^4 alleles is primarily due to the directional trends observed earlier.

Five other variable loci were observed in D. engyochracea. These include EST-2, EST-3, LAP-2, ODH and PGM. Esterase-2 gene frequencies are given in Table 24. It is evident that Kipuka Ki is not in Hardy-Weinberg equilibrium, since only one low-frequency phenotype class (phenotype 23) exists at this locus and it is low in frequency in both populations. A pairwise test for homogeneity indicates that the October 1972 Kipuka Ki sample differs significantly from the March, June and September (1973) collections ($P < 0.001$). The basis for this observation is primarily due to an excess of phenotype 33 and a deficiency of phenotypes 35, 45 and 55 in the October collection. A difference in phenotype frequencies between the March 1973

TABLE 22. Gene frequencies and phenotype observations at the Aldehyde Oxidase (ALDOX) locus in two *D. engyochracea* populations.

Population	Collection	Alleles 2	3	4	5	6	χ^{2A}	χ^{2B}
Kipuka Ki	JUN	.094 ± .04	.179 ± .05	.602 ± .06	.125 ± .04	---	NS	
	SEP	.009 ± .006	.268 ± .04	.719 ± .04	.005 ± .004	---	NS	
	DEC	.080 ± .03	.364 ± .05	.537 ± .06	.019 ± .01	---	44.42(6)***	
	MAR	.071 ± .03	.453 ± .05	.439 ± .05	.038 ± .02	---	20.46(6)***	
	TOTAL	.058 ± .01	.328 ± .03	.575 ± .03	.038 ± .01	---	27.41(6)***	***
Kipuka Puaulu	JUN	.080 ± .03	.191 ± .04	.642 ± .05	.074 ± .03	---	NS	
	SEP	.021 ± .02	.357 ± .06	.614 ± .06	.007 ± .006	.012 ± .01	NS	
	DEC	.016 ± .01	.320 ± .06	.656 ± .06	.008 ± .006	---	NS	
	MAR	.064 ± .03	.363 ± .05	.577 ± .05	---	---	NS	
	TOTAL	.047 ± .01	.307 ± .03	.619 ± .03	.023 ± .01	.001 ± .001	14.95(6)*	***

A Hardy-Weinberg Test (df in parenthesis)

B Contingency Test

NS Not significant

* $P < 0.05$

*** $P < 0.001$

(continued)

TABLE 22. (Concluded) Gene frequencies and phenotype observations at the ALDOX-1 locus in *D. engyochracea*.

		Phenotypes									
Population	Collection	22	23	24	25	33	34	35	44	45	55
Kipuka Ki	JUN	1	1	7	2	3	15	1	22	11	1
	SEP		1	1		4	51		54	1	
	DEC	1	8	3		6	39		22	1	1
	MAR	3	6	3		25	39	1	22	7	
	TOTAL	5	16	14	2	38	144	2	120	20	2
Kipuka Puaulu	JUN	1	2	7	2	4	19	2	36	6	1
	SEP		3			7	32	1	27		
	DEC		2			4	31		26	1	
	MAR	1	5	4		10	41		30		
	TOTAL	2	12	11	2	25	123	3	119	7	1

TABLE 23. Analysis of variance for the allele frequencies at the ALDOX-1 locus in D. engyochracea (populations pooled).

Allele	Source	df	sum squares	mean squares	F
2	Total	668	19.3662		
	Between	7	0.6417	0.0917	3.24**
	Within	661	18.7245	0.0283	
3	Total	668	70.1838		
	Between	7	5.1937	0.7419	7.55**
	Within	661	64.9901	0.0983	
4	Total	668	81.3774		
	Between	7	5.0546	0.7220	6.25**
	Within	661	76.3229	0.1155	
5	Total	668	11.3408		
	Between	7	0.9737	0.1391	8.87**
	Within	661	10.3671	0.0157	

** $P < 0.01$

TABLE 24. Gene frequencies and phenotype observations at the Esterase-2 (EST-2) locus in two D. engyochracea populations.

Population	Collection	Alleles 2	3	4	5	χ^{2A}	χ^{2B}
Kipuka Ki	OCT	---	.590 ± .07	.250 ± .06	.160 ± .05	NS	
	MAR	---	.325 ± .05	.193 ± .04	.455 ± .05	30.35(3)***	
	JUN	---	.432 ± .05	.227 ± .04	.342 ± .04	20.29)3)***	
	SEP	.004 ± .002	.386 ± .04	.246 ± .04	.364 ± .04	15.32(16)***	
	DEC	---	.443 ± .05	.241 ± .04	.316 ± .04	13.54(3)***	
	MAR	---	.425 ± .05	.266 ± .04	.309 ± .04	NS	
	TOTAL	.003 ± .002	.421 ± .02	.240 ± .02	.336 ± .02	33.78(6)***	***
Kipuka Puaulu	OCT	.019 ± .02	.482 ± .10	.389 ± .09	.111 ± .06	NS	
	MAR	---	.367 ± .05	.381 ± .05	.252 ± .04	NS	
	JUN	---	.416 ± .04	.412 ± .04	.172 ± .03	NS	
	SEP	---	.474 ± .05	.339 ± .05	.188 ± .04	NS	
	DEC	.006 ± .01	.500 ± .01	.335 ± .05	.158 ± .04	NS	
	MAR	---	.508 ± .05	.324 ± .04	.168 ± .03	NS	
	TOTAL	.008 ± .005	.445 ± .02	.365 ± .02	.180 ± .02	NS	NS

A Hardy-Weinberg Test (df in parenthesis)

B Contingency Test

NS Not significant

*** $P < 0.001$

(continued)

TABLE 24. (Concluded) Gene frequencies and phenotype observations at the EST-2 locus in D. engyochracea.

		Phenotypes[b]						
Population	Collection	23	33	34	35	44	45	55
Kipuka Ki	OCT		20	15	4	2	6	3
	MAR		1	16	53	6	11	14
	JUN		13	23	52	10	10	9
	SEP	1	9	27	45	4	23	9
	DEC		18	23	42	12	8	11
	MAR		22	22	30	11	16	12
	TOTAL	1	83	126	226	45	74	58
Kipuka Puaulu	OCT	1	7	10	1	4	3	1
	MAR		6	35	36	18	15	3
	JUN		25	41	23	27	18	3
	SEP		22	20	27	19	7	1
	DEC	1	19	22	18	13	5	1
	MAR		31	31	28	19	8	2
	TOTAL	2	110	159	133	100	56	11

b No observations for phenotypes 22, 24 and 25.

and March 1974 samples is also observed ($P < 0.05$). This difference is attributed to a deficiency of phenotype 33 and 44 and an excess of the heterozygote 35 in the March 1973 collection. These observations lead to an increase in the EST-2^3 allele frequency in the October collection and an inflation of the EST-2^5 allele frequency in the March 1973 collection. They do not help much in explaining the Hardy-Weinberg deviations from expected, however, in the March, June, September and December (1973) samples.

An analysis of variance of the primary allele frequencies indicates these to have significant heterogeneity across collections (populations pooled, supplemented Duncan multiple Range Test with total df = 1207 and $P < 0.01$ for each allele). Fluctuations in the gene frequencies between collections are a main contributor to this with no consistent trend observed across the two populations, even though phenotype 35 appears to be declining in frequency after an initial low frequency in the October 1972 sample.

Similar observations to those discussed above can be made for the EST-3 locus (Table 25). Gene frequencies fluctuate in an apparent random manner in both populations and are not necessarily mirrored in each other. For Kipuka Ki, low frequencies of phenotypes 22, 23, 36, 57 and 77 are contributory to the high Chi-square values obtained for the Hardy-Weinberg test for equilibrium. Although low frequency phenotypes are found in high numbers in the Kipuka Puaulu collections (notably 25, 26, 37, 57 and 77), these are in equilibrium with the exception of the June sample ($P < 0.001$, df = 15). For both EST-2 and EST-3 more alleles are found in this study than were detected by Rockwood (1971).

A pairwise contingency test (Table 26) demonstrates many differences between any two samples with respect to phenotype frequencies as well. The observation holds true for Kipuka Ki while Kipuka Puaulu appears to be much more stable. These fluctuations in phenotype frequencies are probably contributory to the significant differences in the variance in allele frequencies (Table 27).

Table 28 provides some evidence that heterozygotes may be at a selective disadvantage in nature since a paucity of these is observed in the samples of both populations in 2/3 of the collections. The September population is not significantly deficient in both populations, nor is the Kipuka Ki March (1972) collection or the December (1972) Kipuka Puaulu collection. This would provide a partial reason why deviation from Hardy-Weinberg expectations is found at the EST-3 locus.

At the LAP-2 locus, the collections are in Hardy-Weinberg equilibrium although the summed data is not (Table 29). For the summed data the phenotype 33 class is

TABLE 25. Gene frequencies and phenotype observations at the Esterase-3 (EST-3) locus in two D. engyochracea populations.

Population	Collection	Alleles 2	3	4	5	6	7	χ^{2A}	χ^{2B}
Kipuka Ki	OCT	---	.319 ± .06	.529 ± .06	.130 ± .04	.022 ± .02	---	73.57(6)***	
	MAR		.116 ± .03	.754 ± .04	.065 ± .02	.065 ± .02	---	31.58(6)***	
	JUN	.009 ± .005	.119 ± .03	.716 ± .04	.106 ± .03	.051 ± .02	---	162.33(10)***	
	SEP	.005 ± .004	.366 ± .05	.489 ± .05	.116 ± .03	.027 ± .02	---	18.53(10)*	
	DEC		.171 ± .03	.421 ± .05	.283 ± .04	.113 ± .03	.012 ± .01	103.73(10)***	
	MAR	.005 ± .004	.168 ± .04	.391 ± .05	.371 ± .05	.035 ± .02	.030 ± .02	109.84(15)***	
	TOTAL	.003 ± .002	.203 ± .02	.553 ± .02	.179 ± .02	.055 ± .01	.001 ± .001	684.95(15)***	***
Kipuka Puaulu	OCT	.106 ± .04	.156 ± .05	.500 ± .06	.221 ± .05	.008 ± .006	.009 ± .006	NS	
	MAR	---	.244 ± .04	.457 ± .05	.235 ± .04	.065 ± .03	---	NS	
	JUN	.035 ± .02	.221 ± .04	.480 ± .04	.126 ± .03	.118 ± .03	.020 ± .01	152.02(15)***	
	SEP	.158 ± .04	.369 ± .06	.247 ± .05	.164 ± .04	.062 ± .03	---	NS	
	DEC	---	.078 ± .03	.303 ± .05	.345 ± .06	.176 ± .05	.098 ± .04	NS	
	MAR	.009 ± .006	.057 ± .03	.528 ± .07	.311 ± .06	.066 ± .03	.029 ± .02	NS	
	TOTAL	.046 ± .01	.202 ± .02	.423 ± .02	.219 ± .02	.087 ± .01	.003 ± .002	368.06(15)***	***

A Hardy-Weinberg Test (df in parenthesis)

B Contingency Test

NS Not significant

* $P < 0.05$

*** $P < 0.001$

(continued)

TABLE 25. (Concluded) Gene frequencies and phenotype observations at the EST-3 locus in D. engyochracea.

Phenotypes[b]																			
22	23	24	25	26	33	34	35	36	37	44	45	46	47	55	56	57	66	67	77
					17	10				27	8	1		5			1		
					6	13	1	1		72	11	7		1	1				
1					8	5	4	1		66	11	8		4			1		
	1				15	39	11	1		30	9	1		2	2		1		
					12	11	6			30	21	9		16	8	1	5		1
	1				12	7	2			13	26			20	5	2	1		2
1	2				70	85	24	3		238	86	26		48	16	3	9		3
6		1			5	8	1			20	11	1		7		1			
					16	16	6	2		30	22	7		11	4		1		
4			1		18	9	4	7		42	17	9	3	3	3	1	5	1	
4	8	5		2	13	8	7	5		9	4	1		6	1				
					1	3	3		3	11	12	4	2	13	6	2	6	3	2
			1			3	3			22	8		1	9	2	1	2	1	
14	8	6	2	2	53	47	24	14	3	134	74	22	6	49	16	5	14	5	2

b No observations for phenotype 27

TABLE 26. Contingency chi-square statistics for the pairwise comparison of collections at the EST-3 locus in two D. engyochracea populations.

		OCT	MAR	JUN	SEP	DEC
MAR	KK	27.59(11)***				
	KP	NS				
JUN	KK	26.91(11)***	NS			
	KP	NS	NS			
SEP	KK	24.99(9)***	49.80(10)***	57.64(9)***		
	KP	NS	48.94(33)*	55.27(37)*		
DEC	KK	25.21(10)**	47.65(9)***	42.29(8)***	50.45(8)***	
	KP	NS	NS	53.59(36)*	68.29(38)*	
MAR	KK	29.22(9)***	68.64(8)***	59.64(7)***	59.61(8)***	NS
	KP	NS	NS	NS	53.77(37)*	NS

KK Kipuka Ki
KP Kipuka Puaulu
NS Not significant
* $P < 0.05$
*** $P < 0.001$

TABLE 27. Analysis of variance for the allele frequencies at the EST-3 locus in *D. engyochracea* (populations pooled).

Allele	source	df	sum squares	mean squares	df
2	Total	1127	19.6735		
	Between	12	2.3558	0.1963	12.64**
	Within	1115	17.3177	0.0155	
3	Total	1127	124.4147		
	Between	12	10.4755	0.8729	8.18**
	Within	1115	118.9392	0.1067	
4	Total	1127	193.7228		
	Between	12	22.3276	1.8606	12.10**
	Within	1115	171.3952	0.1537	
5	Total	1127	115.8084		
	Between	12	11.1523	0.9294	9.90**
	Within	1115	104.6560	0.0938	
6	Total	1127	46.6063		
	Between	12	2.0959	0.1747	4.38**
	Within	1115	44.5104	0.0399	

TABLE 28. Deviations of observed numbers of heterozygotes from the expected in natural populations of D. engyochracea at the EST-3 locus.

Population	Collection	observed	expected	χ^{2A}
Kipuka Ki	OCT	19	56	12.05***
	MAR	34	47	NS
	JUN	29	55	11.82***
	SEP	63	69	NS
	DEC	56	84	9.00**
	MAR	43	68	8.83**
	TOTAL	244	365	39.78***
Kipuka Puaulu	OCT	23	41	7.46**
	MAR	57	86	9.44**
	JUN	55	88	12.00***
	SEP	41	55	NS
	DEC	38	53	NS
	MAR	20	33	4.73*
	TOTAL	234	356	41.47***

A Corrected with Yates correction factor for 1 df

* $P < 0.05$

** $P < 0.01$

*** $P < 0.001$

TABLE 29. Gene frequencies and phenotype observations at the Leucine Aminopeptidase-2 (Lap-2) locus in two D. engyochracea populations.

Population	Collection	Alleles 3	4	5	χ^{2A}	χ^{2B}	33	34	44	45	55
Kipuka Ki	OCT	.007 ± .006	.736 ± .05	.257 ± .05	NS			1	39	24	6
	MAR	---	.587 ± .04	.413 ± .04	NS				44	60	22
	JUN	---	.633 ± .04	.368 ± .04	NS				50	48	19
	SEP	---	.564 ± .04	.436 ± .04	NS				33	57	19
	DEC	.189 ± .04	.638 ± .05	.170 ± .04	NS		7	20	38	19	6
	MAR	---	.661 ± .04	.339 ± .04	NS				50	44	15
	TOTAL	.028 ± .007	.629 ± .02	.343 ± .02	52.95***	***	7	21	254	252	87
Kipuka Puaulu	OCT	---	.794 ± .05	.206 ± .05	NS				45	18	5
	MAR	---	.861 ± .03	.139 ± .03	NS				91	28	3
	JUN	---	.815 ± .03	.185 ± .03	NS				97	39	7
	SEP	.005 ± .01	.779 ± .04	.216 ± .04	NS			1	63	32	6
	DEC	.257 ± .08	.657 ± .08	.086 ± .05	NS		2	14	13	6	
	MAR	---	.851 ± .03	.149 ± .03	NS				92	27	5
	TOTAL	.016 ± .006	.814 ± .02	.170 ± .02	9.07*	***	2	15	401	150	26

a No observations for phenotype 35

A Hardy-Weinberg Test

B contingency test

NS Not significant

* $P < 0.05$

*** $P < 0.001$

the only low-frequency class and is probably negligible in contributing to the significant Chi-square statistic. Heterogeneity is observed between collections in allele LAP-2^3 and LAP-2^5 frequencies and are due to the extremely wide fluctuations observed in these allozymes ($P < 0.01$, df = 1261, populations pooled). A pairwise contingency test between collections within populations shows the December collection to deviate significantly in phenotype frequency from all other collections in Kipuka Ki but not Kipuka Puaulu ($P < 0.05$). Inspection of the phenotype data reveals that the basis for this is a higher than normal occurrence for the phenotypes 33 and 34 which is also reflected in the allele frequencies for LAP-2^3. Fluctuations in the phenotype frequencies appear to be random and are also reflected in the gene frequencies.

At the ODH locus, the October collection for both populations is not in Hardy-Weinberg equilibrium (Table 30). The Chi-square statistic is inflated, however, because of the low expectations in phenotypes 34 and 35 and the low expectations generated for classes 33 and 45 which have zero observations (Kipuka Ki). In Kipuka Puaulu, phenotype 33 has a low expectation and 34 and 35 have zero observations.

A pairwise test for homogeneity generated no significant statistics, indicating no differences in phenotype frequencies between samples. A contingency Chi-square test across collections within populations likewise proved negative.

Of all the loci analyzed in this species, PGM-1 appears to be the most interesting (Table 31). The collections are in Hardy-Weinberg equilibrium with the exception of the March 1973 and September 1973 collections ($P < 0.001$) in Kipuka Ki and the June 1973 collection ($P < 0.05$) in Kipuka Puaulu. The summed data is also not in Hardy-Weinberg equilibrium. Again these observations are due in part to the presence of low-frequency phenotypes, particularly in phenotype classes 33, 34 and 55. The missing observations for phenotype 35 further inflate the Chi-square values because they generate a low-frequency expectation. Contingency tests for the pairwise comparisons and across all samples within populations proved negative.

An analysis of variance of the allele frequencies at this locus indicates significant heterogeneity as present for alleles 3 and 4 (df = 805, $P < 0.01$, populations pooled). The heterogeneity at allele PGM-1^3 is due in part to missing observations in some of the collections; this tended to increase the range of variability for this allele. Heterogeneity in the allele PGM-1^4 frequencies, however, have a different interpretation. The frequencies appear to follow a systematic, diannual trend. The changes are apparent in both populations and are mirrored by opposing changes in the PGM-1^5 allele frequencies.

BLE 30. Gene frequencies and phenotype observations for the Octanol Dehydrogenase-1 (ODH-1) locus in two *D. engyochracea* populations.

		Alleles					Phenotypes[a]				
pulation	Collection	3	4	5	χ^{2A}	χ^{2B}	33	34	35	44	45
puka Ki	OCT	.018 ± .01	.973 ± .02	.009	55.49(3)***			1	1	54	
	MAR	.047 ± .02	.953 ± .02	---	NS			11		107	
	JUN	.058 ± .02	.942 ± .02	---	NS			13		99	
	SEP	---	.983 ± .02	.017 ± .01	NS					28	1
	DEC	.047 ± .02	.953 ± .02	---	NS			12		115	
	MAR	.031 ± .02	.969 ± .02	---	NS			6		90	
	TOTAL	.041 ± .01	.957 ± .01	.002 ± .001	11.67(3)***	NS		43	1	493	1
puka Puaulu	OCT	.018 ± .02	.964 ± .03	.018 ± .01	55.02(3)***		1			52	
	MAR	.010 ± .01	.990 ± .01	---	NS			2		99	2
	JUN	.035 ± .02	.962 ± .02	.004 ± .003	NS			9		120	
	SEP	.010 ± .01	.990 ± .01	---	NS			2		100	
	DEC	.006 ± .005	.987 ± .02	.006 ± .005	NS			1		77	
	MAR	---	.994 ± .01	.006 ± .005	NS					86	
	TOTAL	.014 ± .01	.981 ± .01	.005 ± .002	NS	NS	1	14		534	2

No observations for phenotype 55

Hardy-Weinberg Test (df in parenthesis)

Contingency test

; Not significant

** P < 0.001

BLE 31. Gene frequencies and phenotype observations for the Phosphoglucomutase-1 (PGM-1) locus in two D. engyochracea populations.

		Alleles					Phenotypes[a]				
pulation	Collection	3	4	5	χ^{2A}	χ^{2B}	33	34	44	45	55
puka Ki	OCT	.042 ± .04	.845 ± .07	.104 ± .06	NS			2	18	3	1
	MAR	---	.945 ± .02	.054 ± .02	24.67(1)***				102	6	3
	JUN	.031 ± .02	.856 ± .04	.113 ± .04	NS			5	58	16	1
	SEP	.035 ± .02	.882 ± .03	.083 ± .03	33.76(3)***		2	4	92	13	3
	DEC	---	.941 ± .02	.059 ± .02	NS				90	10	1
	MAR	---	.889 ± .10	.111 ± .10	NS				7	2	
	TOTAL	.020 ± .01	.904 ± .01	.076 ± .01	46.71(3)***	NS	2	11	367	50	9
puka Puaulu	OCT	.023 ± .02	.895 ± .05	.081 ± .04	NS			2	35	5	1
	MAR	.004 ± .001	.956 ± .02	.039 ± .02	NS			1	95	6	1
	JUN	.016 ± .01	.853 ± .04	.132 ± .03	9.34(3)*			3	72	15	5
	SEP	.010 ± .01	.912 ± .04	.078 ± .03	NS			1	42	8	
	DEC	---	.954 ± .03	.047 ± .03	NS				39	4	
	MAR	---	.887 ± .06	.113 ± .06	NS				24	7	
	TOTAL	.022 ± .01	.898 ± .01	.078 ± .01	11.10(3)*	NS		7	307	45	7

No observations for phenotype 35

Hardy-Weinberg Test

Contingency Chi-square

Not significant

$P < 0.05$

* $P < 0.001$

An examination of Table 32 shows that D. engyochracea has an average population heterozygosity of 0.128 in Kipuka Ki and 0.116 in Kipuka Puaulu. As in D. mimica, the Kipuka Ki population is slightly more variable. The locus $\bar{h}$ estimates are higher, on the average, in Kipuka Ki than Kipuka Puaulu with the exception of the EST-3 locus. The same trend was noticed in the D. mimica populations and should be expected here if current genetic theory concerning heterozygote advantage under less stable environmental conditions holds true. Six of 20 loci had significant levels of variability in D. engyochracea (= 30% of loci were variable).

DISCUSSION

Results of genetic analysis of D. mimica and D. engyochracea populations may be summarized as follows. First, extensive genetic variability is present in D. mimica. The variability is expressed in the levels of heterozygosity present ($\bar{H}$ = 0.194, Kipuka Ki; $\bar{H}$ = 0.189, Kipuka Puaulu) and the number of enzyme loci detected in this study as polymorphic (accepting as polymorphic any locus where the most common allele occurs at frequencies less than 95% in frequency; 10 of 21 or 47.6% of such loci are polymorphic in D. mimica).

The analysis shows similar though lower levels of genetic variability present for D. engyochracea. Here, heterozygosity levels for Kipuka Ki = 0.128 and for Kipuka Puaulu = 0.116. Of 20 gene-enzyme loci analyzed in this species, 6 were polymorphic, indicating that 30% of the genes in this species may be variable. Such an indication assumes that gene-enzyme loci are representative of the entire genome.

It is of interest to note that the pattern of genetic variability at the loci studied in both species follows that described by Johnson (1974, see also Table 4). It provides further evidence for Johnson's hypothesis that there exists some functional significance in enzyme polymorphism which is displayed when the enzymes are classified by substrate or by their regulatory role (Table 33).

The analysis of the variability reveals significant heterogeneity between collections in phenotype frequencies exists at some loci. In both populations of D. mimica LAP-2, EST-2, and ALDOX-1 display heterogeneity. In only Kipuka Ki populations, IDH is heterogenous in phenotype frequency while in Kipuka Puaulu LAP-1 and APH-3 are. PGM-1, HK-3, ACPH-1 and ODH-1 are homogenous in both populations with respect to phenotype frequencies.

In D. engyochracea, both populations display heterogeneity in phenotype frequencies at the ALDOX-1, EST-3 and LAP-2 loci. In Kipuka Ki, EST-2 has significant heterogeneity while PGM-1 and ODH-2 are homogenous with respect to phenotype

TABLE 32. Locus and population heterozygosity estimates for six polymorphic loci in D. engyochracea.[a]

Population	Month	PGM-1	EST-2	ODH-1	LAP-2	ALDOX-1	EST-3	$\bar{H}$
Kipuka Ki	OCT	0.259	0.564	0.035	0.392	---[b]	0.602	0.097
	MAR	0.103	0.632	0.089	0.485	---[b]	0.409	0.090
	JUN	0.253	0.646	0.109	0.466	0.583	0.500	0.128
	SEP	0.214	0.658	0.033	0.492	0.443	0.618	0.123
	DEC	0.111	0.646	0.090	0.569	0.572	0.701	0.134
	MAR	0.197	0.653	0.060	0.448	0.642	0.678	0.139
	Average	0.175	0.652	0.078	0.501	0.556	0.604	0.128
Kipuka Puaulu	OCT	0.190	0.605	0.070	0.327	---[b]	0.666	0.098
	MAR	0.083	0.657	0.010	0.239	---[b]	0.675	0.088
	JUN	0.256	0.628	0.067	0.302	0.538	0.689	0.124
	SEP	0.162	0.626	0.020	0.347	0.493	0.748	0.120
	DEC	0.090	0.680	0.010	0.555	0.467	0.741	0.127
	MAR	0.200	0.609	0.012	0.254	0.570	0.617	0.113
	Average	0.166	0.632	0.037	0.352	0.518	0.681	0.116

a See Table 3 for description of $\bar{h}$ and $\bar{H}$.

b Not analyzed due to technical difficulties.

TABLE 33. Comparative functional classification of enzyme variability in *D. mimica* and *D. engyochracea*.

Class of Enzyme	Others[a]	D. mimica	D. engyochracea
Variable substrate	.240	.505	.266
Regulatory	.190	.086	.073
Non-regulatory	.067	.072	.000

[a] Pooled, from other Drosophila studies, see Johnson, 1974.

frequencies over time.

Generally, Kipuka Ki populations of D. engyochracea are less often in genetic equilibrium than Kipuka Puaulu populations. D. mimica, on the other hand, is equally as often in Hardy-Weinberg equilibrium in both populations. Examination of the data reveals, however, that EST-2, LAP-1 and LAP-2 are contributing the most to this observation in D. mimica. In D. engyochracea, EST-2 and EST-3 contribute the most to this situation.

When examined from the viewpoint of time, it appears that for Kipuka Ki populations of D. mimica, June and December are times when the most genetic disequilibrium in phenotype frequencies is experienced. In Kipuka Puaulu populations, a trend toward bi-annual disequilibrium is also observed during December-January and July-June. For D. engyochracea Kipuka Ki populations, December contains the highest deviations from Hardy-Weinberg equilibrium with 4 loci involved: ALDOX-1, EST-2, EST-3 and LAP-2. The Kipuka Puaulu population, on the other hand, appears much more stable with no trends as to which loci are at disequilibrium or what time of the year the most loci are in deviation from Hardy-Weinberg expectations.

Although certain biases exist in the discrimination ability of the Chi-square tests, the observed heterogeneity in allele frequencies discussed above is supported by analysis of variance at polymorphic gene loci. Although not significant, the heterozygosity data support the thesis that Kipuka Ki populations are more variable than Kipuka Puaulu populations. In addition, Kipuka Ki populations are more dynamic at the genetic level.

It is of particular interest to note that the heterogeneity in allele frequencies in both populations of D. engyochracea and D. mimica is essentially of three types. These are long term directional (PGM-1, IDH-1 and EST-2 in D. mimica, PGM-1 in D. engyochracea), short-term cyclical (ACPH, D. mimica) and those manifesting no consistent pattern.

The slight differences in variability noted between the two Kipukas is in accordance with the slightly more xeric conditions of Kipuka Ki. These slight divergences have not resulted in sharply divergent gene pools, however, following the geological formation of the Kipukas. This point is borne out by the fact that at all monomorphic loci the same allele is fixed in both localities. In addition, polymorphic loci have the same alleles in approximately the same frequencies with the only exceptions being rare alleles occurring at less than 1%. Such alleles are not present in all samples even within Kipukas and their detection is highly dependent on sample size. These facts can be considered as evidence against gene pool

differentiation.

Another piece of evidence to favor non-differentiation of gene pools is the fact that genetic shifts in one population are usually mirrored in the other. This, along with the occurrence of the same alleles in both Kipukas, implies that similar forces are acting on the genes which lie at the basis of the enzyme systems in question.

The value of the present study lies in the exposure of two important points, the finding of extensive genetic variability in both species equivalent to that found in continental or cosmopolitan Drosophila species, and the mode of gene responses at specific loci. The first indicates that finite and isolated populations which have experienced severe reductions in size in the past can still maintain large levels of genetic variability. It is not clear whether present levels have arisen since formation of the Kipukas or have been present all along. The populations must have been much larger in the past; correspondingly they were also much younger. The extent these two factors have had on present levels of variability is conjectural at best. The maintenance of the present levels of variability must be dependent upon some selective factors at loci demonstrating directional or cyclical changes; they cannot be due to migration as is possible in previous studies of Hawaiian Drosophila (Johnson et al., 1975, Steiner et al., 1974) first because these are the only two populations known of both species and second, because the genetic events occur in both populations. Although the EST-2, LAP-2 and APH-3 loci of D. mimica are extremely polymorphic, Levene's hypothesis of multiple-niche effect on genetic variation may apply to D. mimica since it breeds in at least 6 different substrates (Heed, 1968). Thus the evidence is suggestive of selection at all electrophoretic loci studied in this species.

The same is not true at those polymorphic loci which display stochastic and undeterministic changes. In D. engyochracea only PGM-1 demonstrates directional changes in gene frequencies. The other 4 loci display changes which cannot be related to knowledge of either engyochracea's ecology, ethology or what is known of its environment. It is obvious that more indepth studies are needed to determine the factors contributing to maintenance of genetic polymorphism in these species.

One way of getting at the question of the extent that neutrality affects maintenance at polymorphic protein loci in engyochracea would be to determine if assumptions predicted by non-Darwinian theory are met by the data. Kimura and Crow (1964) have calculated the effective number of neutral alleles (ne) expected in randomly mated, finite populations of various sizes. Recently unpublished

experiments have indicated that the size of the Kipuka Ki engyochracea population may be about 25,000 individuals in the sample area. Although further work may result in an alteration of this figure it has nevertheless been used in the calculations which follow. Kimura and Crow's estimate for a population of this size, which is dependent on mutation rate of 10^{-6}, is ne = 1.04 - 1.004. For each polymorphic locus in D. engyochracea, these estimates are too small (Table 34), even when the effective number is calculated for individual loci via the formula

$$ne = {}^{1}/_{\Sigma p_i^2}$$

where p_i is the frequency of the ith allele at a locus. At the ODH and LAP-2 loci, the number of neutral alleles expected is approached when the observed number of alleles is adjusted for low-frequency (rare) alleles. We cannot say for certain, then, that selection is maintaining variability at these two loci. The fact that they are not in Hardy-Weinberg equilibrium at all times, however, is indicative that selective events may be associated with these loci.

Table 34 also indicates that the average expected number of neutral alleles at a locus is 1.19 as calculated from the adjusted formula of Ohta and Kimura (1973) where μ assumes a value of 10^{-7}. This also is smaller than the actual number of observed alleles, even when the latter is adjusted for rare alleles. Ayala et al. (1974), in similar calculations for species of the Drosophila willistoni group, found that the observed numbers of alleles was much less than the expected which is opposite to our findings. This discrepancy is accounted for by noting that vast differences in population size exist here: in the case of the D. willistoni group, populations reach cosmopolitan types of distribution and may number in the many millions. In our case, we are dealing with an isolated and finite population. Ohta and Kimura's formula is dependent upon population size and it is interesting to observe that in neither Ayala's case nor ours do the predictions fit the observations. This suggests that the alleles being observed in both cases are experiencing some type of selection pressure; that is they may serve some adaptive function with respect to their gene pools they are drawn from. The higher number of observed neutral alleles per locus in the Hawaiian species are compared to the D. willistoni group and the expected ne is seen even when all loci are taken into account (obs. no. = 1.85, obs. no. occurring over 5% in frequency = 1.55). The reason for this is unknown, certainly these species are much younger than those comprising the D. willistoni group. This, coupled with limited population size, would suggest that the number of observed alleles should be lower in this Hawaiian species. We cannot

TABLE 34. The number of alleles observed at the polymorphic loci found in D. engyochracea and the number of neutral alleles expected (see Crow and Kimura, 1970 for a discussion of the latter).

Locus	Observed No. of Alleles	Observed No. of Alleles occurring over 5%	Expected No. of[a] Neutral Alleles
ALDOX	4	3	2.26
EST-2	4	3	2.58
EST-3	6	4	2.62
LAP-2	3	4	1.94
PGM	3	2	1.22
ODH	3	1	1.09
Average n_e/locus	3.83	2.83	1.19[b]

a individual ne = $1/\Sigma Pi^2$ (Kimura and Crow, 1964)

b average ne = $\sqrt{1 + 8Nu}$ (Ohta and Kimura, 1973).

suggest reasons why this is not the case, certainly it has important implications with respect to the rapid and phenomenal rates of speciation observed to occur in the Hawaiian Drosophila.

The environment occupied by these species appears to be stable within limits (Bridges and Carey, 1973), however it stands to reason that these bounds may be overreached at rare intervals. It is possible that at least some of this apparently excess variation may serve as a genetic resource during times of environmental stress and be carried along in situ at other times in a largely neutral state. Rare occasions of disequilibrium in the environment would prevent loss of such drifting alleles, and in the defined areas occupied by small and isolated populations these could become evolutionary parameters to be reckoned with where the continued existence of the gene pool was at stake. Although we hesitate to say that the genetic polymorphism observed in this case has evolved and is acting strictly as an answer to environmental disequilibrium in an otherwise fairly homogenous environment, we point out that it not only provides one way of handling such environmental "overruns" but serves as a source of variation which may contribute in some manner to the high rates of speciation observed in these species. Further proof for such a hypothesis can come from the demonstration of higher levels of genic variability in small, finite, endemic populations of highly-speciating taxa than observed in similar populations of low-speciating taxa.

In this survey, we have demonstrated that extensive genic variability can exist in isolated, finite and endemic Hawaiian Drosophilid populations. Extensive heterozygosity is present even in a species which displays a narrow breeding niche (D. engyochracea). Although these populations cannot be considered "small" in any sense, they do occupy an environment which is not without some heterogeneity at least with respect to moisture levels. After careful scrutiny, we suggest that the genic variability found in these species may be maintained largely by natural selection and is of some adaptive value. We will present further evidence to support this thesis in future reports.

ACKNOWLEDGEMENTS

We wish to thank the staff of the Hawaii IBP and the Hawaiian Drosophila Project for their assistance in various phases of this project. Peter Galloway and Lee Annest assisted on several field trips. Gwen Arakaki and Alan Ohta provided technical assistance in the lab. Discussions with Drs. J. N. Ahearn, K. Y. Kaneshiro, F. C. do Val and W. E. Johnson proved invaluable at various stages of the project. Dr. W. E. Johnson provided serious comment on portions of this manuscript. The research reported here was submitted to the Department of Genetics, University of Hawaii, in partial fulfillment for the degree of Doctor of Philosophy in Genetics awarded to W. W. M. S. This research was supported by NSF grants GB-23230, GB-27586 and GB-29288.

REFERENCES

Alder, H. L. and E. B. Roessler. 1972. Introduction to probability and statistics. W. H. Freeman and Co., S. F., Calif. 5th edition.

Ayala, F. J., J. R. Powell and Th. Dobzhansky. 1971. Polymorphisms in continental and island populations of Drosophila willistoni. Proc. Nat. Acad. Sci. USA 68:2480-2483.

Ayala, F. J., M. C. Tracey, L. G. Barr, J. F. McDonald and S. Perez-Salas. 1974. Genetic variation in natural populations of five Drosophila species and the hypothesis of the selective neutrality of protein polymorphisms. Genetics 77:343-384.

Beardmore, J. and L. Levine. 1963. Fitness and environmental variation. I. A study of some polymorphic populations of Drosophila pseudoobscura. Evolution 17:121-129.

Bridges, K. W. and G. V. Carey. 1973. The climate of the IBP sites on Mauna Loa, Hawaii. US/IBP Island Ecosystems IRP Tech. Rep. #22. 141 p.

Carson, H. L., F. E. Clayton and H. D. Stalker. 1967. Karyotypic stability and speciation in Hawaiian Drosophila. Proc. Nat. Acad. Sci. USA 57:1280-1285.

Carson, H. L. and J. E. Sato. 1969. Microevolution within three species of Hawaiian Drosophila. Evolution 23:493-501.

Carson, H. L., D. E. Hardy, H. T. Spieth and W. S. Stone. 1970. The evolutionary biology of the Hawaiian Drosophilidae, in Essays In Evolution And Genetics In Honor of Theodosius Dobzhansky (M. K. Hecht and W. C. Steere, ed.). Appleton-Century-Croft, N. Y. pp. 437-543.

Crow, J. F. and M. Kimura. 1970. An Introduction to Population Genetic Theory. Harper and Row, N. Y.

Duncan, D. B. 1955. Multiple range and multiple F-test. Biometrics 11:1042.

Hardy, D. E. 1965. Insects of Hawaii, Vol. 12; Diptera; Cyclorrhapha II, Series Schizophora, Section Acalypterae I. Family Drosophilidae. Univ. Hawaii Press, Honolulu.

Heed, W. B. 1968. Ecology of the Hawaiian Drosophilidae. Studies in Genetics IV. Univ. Tex. Publ. 6818:387-420.

Heed, W. B. and J. S. Russell. 1971. Phylogeny and population structure in island and continental species of the Cardini group of Drosophila studied in inversion analysis. Studies in Genetics VI. Univ. Tex. Publ. 7103:91-130.

Johnson, F. M., K. Kojima and M. R. Wheeler. 1969. Isozyme variation in Drosophila island populations. II. An analysis of Drosophila ananassae populations in the Samoan, Fijian and Philippine Islands. Studies in Genetics V. Univ. Tex. Publ. 6918:187-205.

Johnson, F. M. 1971. Isozyme polymorphisms in Drosophila ananassae: genetic diversity among island populations in the South Pacific. Genetics 68:77-95.

Johnson, G. B. 1974. Enzyme polymorphism and metabolism. Science 184:28-37.

Johnson, W. E., H. L. Carson, K. Y. Kaneshiro, W. W. M. Steiner and M. M. Cooper. 1975. Genetic variation in Hawaiian Drosophila II. Allozymic differentiation in the D. planitibia subgroup. In Press.

Kambysellis, M. P. and W. B. Heed. 1971. Studies of oogenesis in natural populations of Drosophilidae. I. Relation of ovarian development and ecological habitats of the Hawaiian species. Am. Nat. 105:31-49.

Kambysellis, M. P. 1974. Effects of microclimatic changes in oogenesis of Drosophila mimica. US/IBP Island Ecosystems IRP Tech. Rep. #39. 58 p.

Kaneshiro, K. Y. 1969. A study of the relationships of Hawaiian Drosophila species based on male genitalia. Studies in Genetics V. Univ. Tex. Publ. 6918:55-70.

Kimura, M. and J. F. Crow. 1964. The number of alleles that can be maintained in a finite population. Genetics 49:725-738.

Levene, H. 1953. Genetic equilibrium when more than one ecological niche is available. Am. Nat. 87:331-333.

Lindgren, B. W. 1962. Statistical Theory. MacMillan Co., N. Y.

Montgomery, S. L. 1972. Comparative Breeding Site Ecology and the Adaptive Radiation of Picture-winged Drosophilidae (Diptera: Drosophilidae) in Hawaii. Univ. Hawaii Masters Thesis, Entomology.

Mueller-Dombois, W. and C. H. Lamoureux. 1967. Soil-vegetation relationships in Hawaiian Kipukas. Pac. Sci. 21:286-299.

Ohta, T. and M. Kimura. 1973. A new model for estimating the number of electrophoretically detectable alleles in a finite population. Genetics 74:s201.

Prakash, S., R. C. Lewontin and J. C. Hubby. 1969. A molecular approach to the study of genic heterozygosity in natural populations. IV. Patterns of genic variation in central, marginal and isolated populations of Drosophila pseudoobscura. Genetics 61:841-858.

Richardson, R. H. 1974. Effects of dispersal, habitat selection and competition on a speciation pattern of Drosophila endemic to Hawaii. in: Genetic Mechanisms of Speciation in Insects. M. J. D. White, ed. Australia and New Zealand Book Co., Sydney. pp. 140-164.

Rockwood, E. S. 1969. Enzyme variation in natural populations of Drosophila mimica. Studies in Genetics V. Univ. Tex. Publ. 6918:111-132.

Rockwood, E. S., C. G. Kanapi, M. R. Wheeler and W. S. Stone. 1971. Allozyme changes during the evolution of the Hawaiian Drosophila. Studies in Genetics VI. Univ. Tex. Publ. 7103:193-212.

Slobodkin, L. and H. Sanders. 1969. On the contribution of environmental predictability to species diversity. Brookhaven Symp. Biol. 22:82-95.

Snedecor, G. W. and W. G. Cochran. 1969. Statistical Methods. Univ. Iowa Press, Ames, Iowa. 6th edition.

Spieth, H. T. 1966. A method for transporting adult Drosophila. DIS 41:197.

Spieth, H. T. 1968. Evolutionary implications of sexual behavior in Drosophila, in Evolutionary Biology (Th. Dobzhansky, M. K. Hecht and W. C. Steere, eds.). pp. 157-193.

Steiner, W. W. M. and W. E. Johnson. 1973. Techniques for electrophoresis of Hawaiian Drosophila. US/IBP Island Ecosystems IRP Tech. Rep. #30. 22 p.

Steiner, W. W. M., W. E. Johnson and H. L. Carson. 1973. Molecular differentiation in D. grimshawi. DIS 50:100-101.

Steiner, W. W. M. 1975. Genetics of five allozyme loci in a Hawaiian Drosophila. Submitted to DIS (Drosophila Information Service).

TECHNICAL REPORTS OF THE US/IBP ISLAND ECOSYSTEMS IRP

(Integrated Research Program)

*No. 1 Hawaii Terrestrial Biology Subprogram. First Progress Report and Second-Year Budget. D. Mueller-Dombois, ed. December 1970. 144 p.

*No. 2 Island Ecosystems Stability and Evolution Subprogram. Second Progress Report and Third-Year Budget. D. Mueller-Dombois, ed. January 1972. 290 p.

*No. 3 The influence of feral goats on koa (Acacia koa Gray) reproduction in Hawaii Volcanoes National Park. G. Spatz and D. Mueller-Dombois. February 1972. 16 p.

*No. 4 A non-adapted vegetation interferes with soil water removal in a tropical rain forest area in Hawaii. D. Mueller-Dombois. March 1972. 25 p.

*No. 5 Seasonal occurrence and host-lists of Hawaiian Cerambycidae. J. L. Gressitt and C. J. Davis. April 1972. 34 p.

*No. 6 Seed dispersal methods in Hawaiian Metrosideros. Carolyn Corn. August 1972. 19 p.

*No. 7 Ecological studies of Ctenosciara hawaiiensis (Hardy) (Diptera: Sciaridae). W. A. Steffan. August 1972. 7 p.

*No. 8 Birds of Hawaii Volcanoes National Park. A. J. Berger. August 1972. 49 p.

*No. 9 Bioenergetics of Hawaiian honeycreepers: the Amakihi (Loxops virens) and the Anianiau (L. parva). R. E. MacMillen. August 1972. 14 p.

*No. 10 Invasion and recovery of vegetation after a volcanic eruption in Hawaii. G. A. Smathers and D. Mueller-Dombois. September 1972. 172 p.

*No. 11 Birds in the Kilauea Forest Reserve, a progress report. A. J. Berger. September 1972. 22 p.

No. 12 Ecogeographical variations of chromosomal polymorphism in Hawaiian populations of Drosophila immigrans. Y. K. Paik and K. C. Sung. February 1973. 25 p.

*No. 13 The influence of feral goats on the lowland vegetation in Hawaii Volcanoes National Park. D. Mueller-Dombois and G. Spatz. October 1972. 46 p.

*No. 14 The influence of SO_2 fuming on the vegetation surrounding the Kahe Power Plant on Oahu, Hawaii. D. Mueller-Dombois and G. Spatz. October 1972. 12 p.

No. 15 Succession patterns after pig digging in grassland communities on Mauna Loa, Hawaii. G. Spatz and D. Mueller-Dombois. November 1972. 44 p.

No. 16 Ecological studies on Hawaiian lava tubes. F. G. Howarth. December 1972. 20 p.

No. 17 Some findings on vegetative and sexual reproduction of koa. Günter O. Spatz. February 1973. 45 p.

No. 18 Altitudinal ecotypes in Hawaiian Metrosideros. Carolyn Corn and William Hiesey. February 1973. 19 p.

No. 19 Some aspects of island ecosystems analysis. Dieter Mueller-Dombois. February 1973. 26 p.

No. 20 Flightless Dolichopodidae (Diptera) in Hawaii. D. Elmo Hardy and Mercedes D. Delfinado. February 1973. 8 p.

* out of print

No. 21 Third Progress Report and Budget Proposal for FY 74 and FY 75. D. Mueller-Dombois and K. Bridges, eds. March 1973. 153 p.

No. 22 Supplement 1. The climate of the IBP sites on Mauna Loa, Hawaii. Kent W. Bridges and G. Virginia Carey. April 1973. 141 p.

No. 23 The bioecology of Psylla uncatoides in the Hawaii Volcanoes National Park and the Acacia koaia Sanctuary. John R. Leeper and J. W. Beardsley. April 1973. 13 p.

No. 24 Phenology and growth of Hawaiian plants, a preliminary report. Charles H. Lamoureux. June 1973. 62 p.

No. 25 Laboratory studies of Hawaiian Sciaridae (Diptera). Wallace A. Steffan. June 1973. 17 p.

No. 26 Natural area system development for the Pacific region, a concept and symposium. Dieter Mueller-Dombois. June 1973. 55 p.

No. 27 The growth and phenology of Metrosideros in Hawaii. John R. Porter. August 1973. 62 p.

*No. 28 EZPLOT: A computer program which allows easy use of a line plotter. Kent W. Bridges. August 1973. 39 p.

No. 29 A reproductive biology and natural history of the Japanese white-eye (Zosterops japonica japonica) in urban Oahu. Sandra J. Guest. September 1973. 95 p.

No. 30 Techniques for electrophoresis of Hawaiian Drosophila. W. W. M. Steiner and W. E. Johnson. November 1973. 21 p.

No. 31 A mathematical approach to defining spatially recurring species groups in a montane rain forest on Mauna Loa, Hawaii. Jean E. Maka. December 1973. 112 p.

*No. 32 The interception of fog and cloud water on windward Mauna Loa, Hawaii. James O. Juvik and Douglas J. Perreira. December 1973. 11 p.

No. 33 Interactions between Hawaiian honeycreepers and Metrosideros collina on the island of Hawaii. F. Lynn Carpenter and Richard E. MacMillen. December 1973. 23 p.

No. 34 Floristic and structural development of native dry forest stands at Mokuleia, N.W. Oahu. Nengah Wirawan. January 1974. 49 p.

No. 35 Genecological studies of Hawaiian ferns: reproductive biology of pioneer and non-pioneer species on the island of Hawaii. Robert M. Lloyd. February 1974. 29 p.

No. 36 Fourth Progress Report and Budget Proposal for FY 1975. D. Mueller-Dombois and K. Bridges, eds. March 1974. 44 p.

No. 37 A survey of internal parasites of birds on the western slopes of Diamond Head, Oahu, Hawaii 1972-1973. H. Eddie Smith and Sandra J. Guest. April 1974. 18 p.

No. 38 Climate data for the IBP sites on Mauna Loa, Hawaii. Kent W. Bridges and G. Virginia Carey. May 1974. 97 p.

No. 39 Effects of microclimatic changes on oogenesis of Drosophila mimica. Michael P. Kambysellis. May 1974. 58 p.

No. 40 The cavernicolous fauna of Hawaiian lava tubes, Part VI. Mesoveliidae or water treaders (Heteroptera). Wayne C. Gagné and Francis G. Howarth. May 1974. 22 p.

* out of print

No. 41 Shade adaptation of the Hawaiian tree-fern (Cibotium glaucum (Sm.) H. & A.). D. J. C. Friend. June 1974. 39 p.

No. 42 The roles of fungi in Hawaiian Island ecosystems. I. Fungal communities associated with leaf surfaces of three endemic vascular plants in Kilauea Forest Reserve and Hawaii Volcanoes National Park, Hawaii. Gladys E. Baker, Paul H. Dunn and William A. Sakai. July 1974. 46 p.

No. 43 The cavernicolous fauna of Hawaiian lava tubes, Part VII. Emesinae or thread-legged bugs (Heteroptera: Redvuiidae). Wayne C. Gagné and Francis G. Howarth. July 1974. 18 p.

No. 44 Stand structure of a montane rain forest on Mauna Loa, Hawaii. Ranjit G. Cooray. August 1974. 98 p.

No. 45 Genetic variability in the Kilauea Forest population of Drosophila silvestris. E. M. Craddock and W. E. Johnson. September 1974. 39 p.

No. 46 Linnet breeding biology on Hawaii. Charles van Riper III. October 1974. 19 p.

No. 47 The nesting biology of the House Finch, Carpodacus mexicanus frontalis (Say), in Honolulu, Hawaii. Lawrence T. Hirai. November 1974. 105 p.

No. 48 A vegetational description of the IBP small mammal trapline transects - Mauna Loa Transect. James D. Jacobi. November 1974. 19 p.

No. 49 Vegetation types: a consideration of available methods and their suitability for various purposes. Dieter Mueller-Dombois and Heinz Ellenberg. November 1974. 47 p.

No. 50 Genetic structure and variability in two species of endemic Hawaiian Drosophila. William W. M. Steiner and Hampton L. Carson. December 1974. 66 p.